

The α -Substitution of Chalcones as a Tool to Modulate the Reactivity and Biological Activity

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Nafisah Al-Rifai

aus

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Diese Arbeit wurde angeleitet von: PD Dr. Sabine Amslinger

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Prüfungsausschuss:

- Prof. Dr. Sigurd Elz (Vorsitzender)
- PD Dr. Sabine Amslinger
- Prof. Dr. Burkhard König
- Prof. Dr. Jörg Heilmann

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ABBREVIATIONS

ABP	Activity-based probe
ABPP	Activity-based protein profiling
Ac ₂ O	Acetic anhydride
AcOH	Acetic acid
aq.	Aqueous
ARE	Antioxidant response elements
ATP	Adenosine triphosphate
ATR	Attenuated total reflectance
COX-2	Cyclooxygenase -2
Cul3	Cullin 3
DAD	Diode-array detector
DMAP	4-Dimethylaminopyridine
DMP	Dess-Martin periodinane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EG	Ethylene glycol
EI	Electron ionization
eNOS	Endothelial NO synthase
eq.	Equivalent
ESI	Electron spray ionization
ES-MS	Electron spray-mass spectrometry
Et ₂ O	Diethyl ether
EtOH	Ethanol
FCS	Fetal calf serum
GC-MS	Gas chromatography-mass spectrometry
GSH	Glutathione
HC	2'-Hydroxy-3,4,4'-Trimethoxychalcone
HMDS	Hexamethyldisilazane
HO-1	Heme oxygenase-1
HOMO	Higher occupied molecular orbitals
HPLC	High pressure liquid chromatography

HRMS	High resolution mass spectrometry
IKK	IKK: I κ B kinase
iNOS	Inducible NO synthase
IR	Infrared
I κ B α	Inhibitor of kappa B, α
Keap1	Kelch-like ECH-associated protein1
KHMDS	Potassium hexamethyldisilazide
LC-MS	Liquid chromatography-Mass spectrometry
LDA	Lithium diisopropylamine
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
LUMO	Lower unoccupied molecular orbitals
MeCN	Acetonitrile
MeOH	Methanol
MFSDA	Methyl fluorosulfonyldifluoroacetate
MS	Mass spectrometry
MS	Molecular sieves
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NED	<i>N</i> -1-Naphthylethylenediamine
NF- κ B	Nuclear factor-kappa B
NMP	<i>N</i> -Methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
nNOS	Neuronal NO synthase
NOS	Nitric oxide synthase
NQO1	NAD(P)H:quinine oxireductase-1
Nrf2	Nuclear factor-erythroid-2-related factor 2
PCC	Pyridinium chlorochromate
PCR	Polymerase Chain Reaction
pHC	2'-O-Isopropyl (protected)-3,4,4'-Trimethoxychalcone
rt	Room temperature
SDS	Sodium dodecyl sulfate
THF	Tetrahydrofuran
TLC	Thin-Layer chromatography
TMC	2',3,4,4'-Tetramethoxychalcone

TNF	Tumor necrosis factor
TRIS-HCl	Tris(hydroxymethyl)aminomethane hydrochloride

1. INTRODUCTION

1.1. Electrophiles and inflammation

There are many bioactive natural products with different structures that affect their targets by their electrophilic nature. These electrophilic natural products convey diverse biological activities, such as antifungal, antimetabolic, or antitumor activity. Many others play important roles in fighting inflammation. Michael acceptor systems such as α,β -unsaturated aldehydes, ketones, amides, γ -lactones and δ -lactones (Figure 1), as well as ring strained cyclic molecules such as epoxides, aziridines, β -lactones and β -lactams are some examples.¹

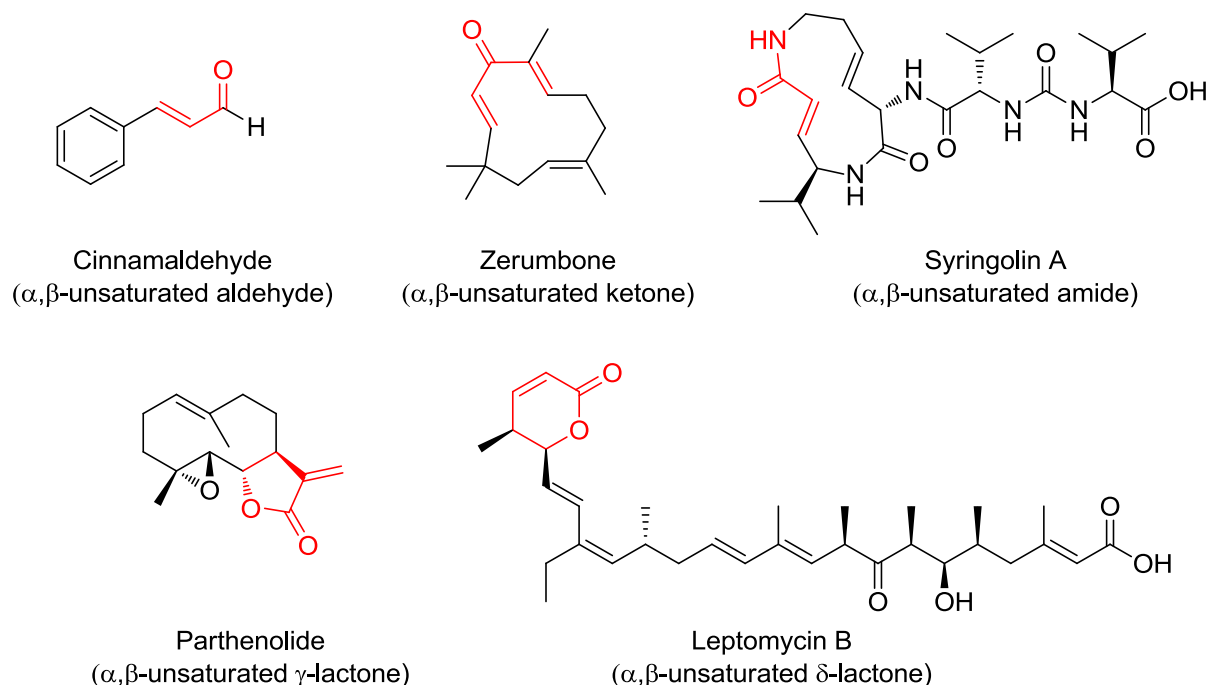


Figure 1: Some natural α,β -unsaturated compounds.¹

The activity of the α,β -unsaturated carbonyl compounds is mostly based on their Michael acceptor activity, *i.e.* adding nucleophiles to the electrophilic β -position of their enone system.² Through this activity, α,β -unsaturated carbonyl compounds trigger the activation or inhibition of anti- and proinflammatory pathways, where reactive sulfhydryl groups of cysteine residues in proteins play a major role in transforming chemical reactivity into a biological activity.³ Depending on their structures, α,β -unsaturated carbonyl systems may have other types of reactivity like radical scavenging, double bond isomerization or reductive potential which are often referred to as antioxidative behavior (Figure 2).²

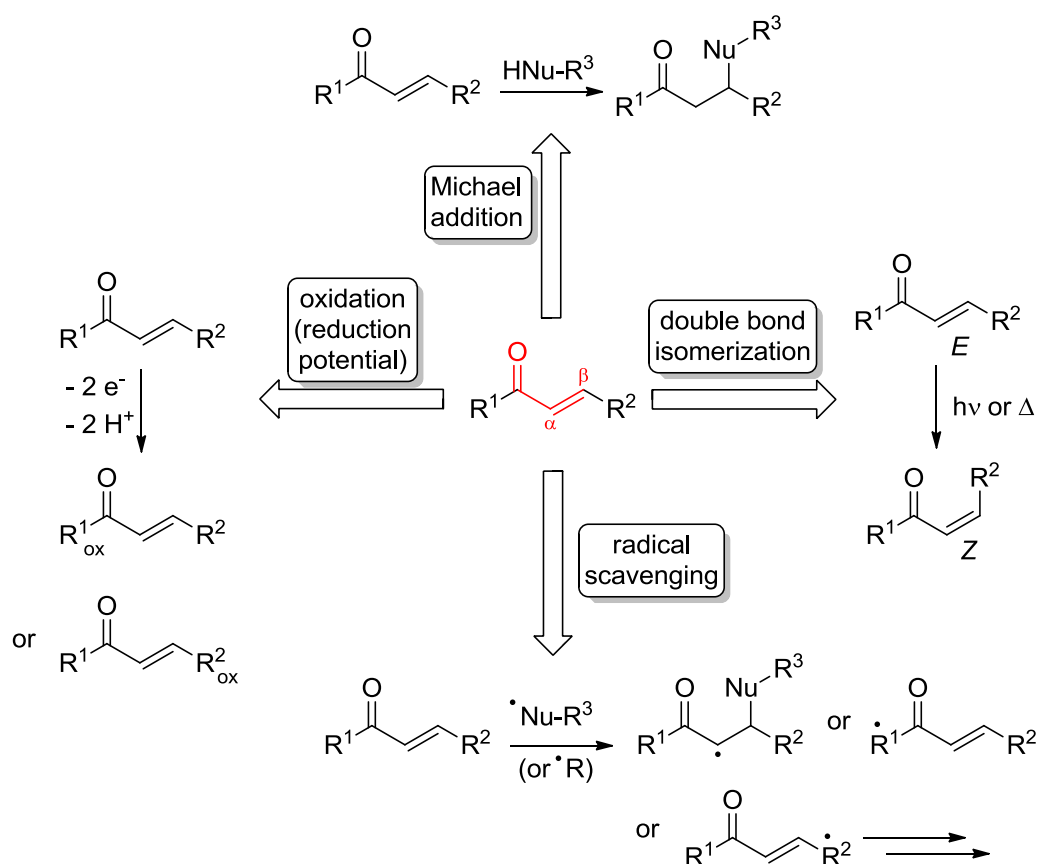


Figure 2: Important reactivities of α,β -unsaturated compounds.²

The activation of the Nrf2-Keap1 pathway and the inhibition of the NF- κ B pathway are examples of cellular defense strategies against inflammation where the thiol-mediated processes play a key role leading to induction of phase 2 enzymes. Nrf2 (nuclear factor-erythroid-2-related factor 2) is a transcription factor which is tightly regulated by a cytosolic repressor protein called Kelch-like ECH-associated protein1 (Keap1). Keap1, in combination with an adaptor component Cul3 (Cullin 3)-based ubiquitin E3 ligase complex, promotes Nrf2 ubiquitination and proteosomal degradation in basal conditions.⁴ As shown in Figure 3, Keap1 can be activated either by oxidation (leading to the formation of disulfide) or by a Michael addition reaction with an electrophile such as an α,β -unsaturated compound. This leads to liberation of the transcriptional factor Nrf2 which then translocates into the nucleus and binds to antioxidant response elements (ARE) leading to expression of anti-inflammatory proteins like heme oxygenase-1 (HO-1).⁵

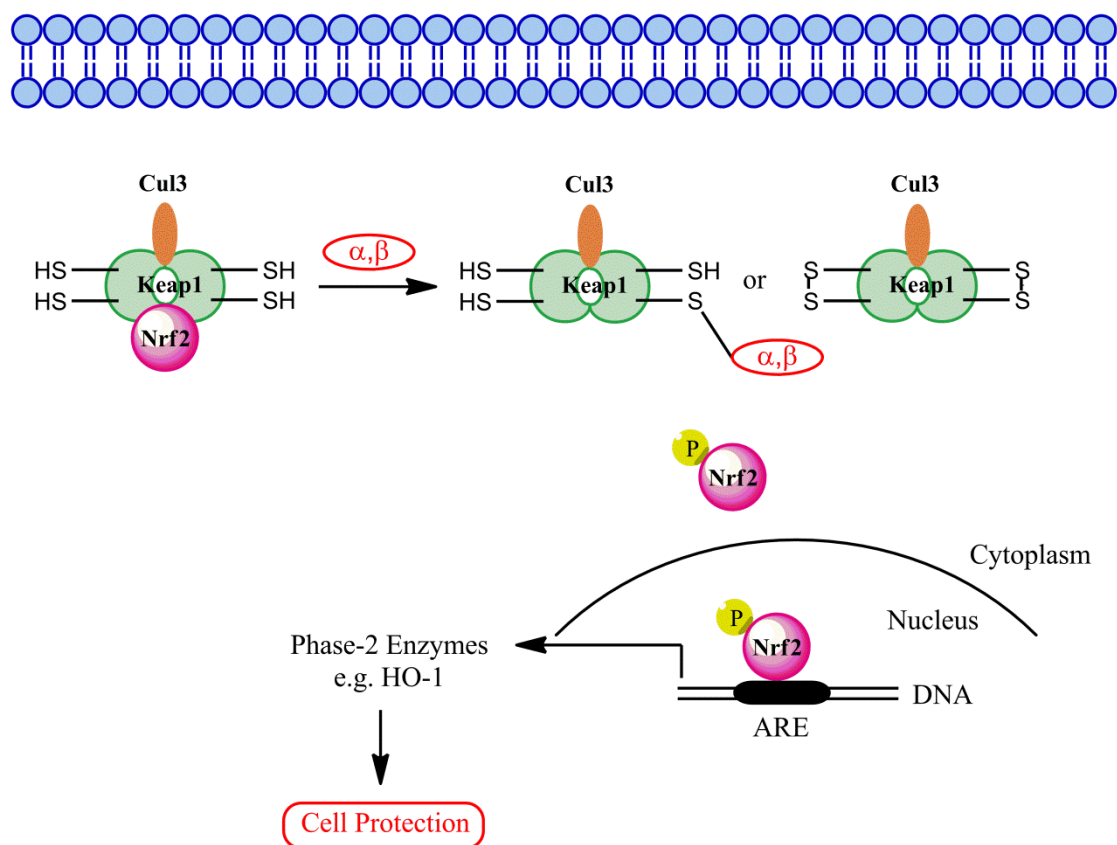


Figure 3: Mechanism of Nrf2-Keap1 activation pathway, (Cul3: Cullin 3; Keap1: Kelch-like ECH-associated protein1; Nrf2: nuclear factor-erythroid-2-related factor 2; α,β : α,β -unsaturated compound; ARE: Antioxidant response element; HO-1: heme oxygenase-1).

On the other hand, the transcriptional factor nuclear factor-kappa B (NF- κ B) is a heterodimer of p65 and p50 proteins. In unstimulated cells, NF- κ B is sequestered in the cytoplasm by binding to inhibitor proteins (I κ Bs) which are themselves controlled by protein kinases (IKKs). Following cell stimulation, IKK activates I κ B α by phosphorylation of its serine residues. The activation of I κ B α leads to its ubiquitination and then degradation which liberates the NF- κ B. The free NF- κ B translocates into the nucleus and binds to target DNA sites resulting in transcription of many proinflammatory genes.⁶ Therefore, the deactivation of NF- κ B, where an alkylation of reactive cysteines at p50 and p65 DNA binding sites or at the regulatory kinase IKK is crucial, is of great importance. It leads to downregulation of proinflammatory factors like tumor necrosis factor (TNF, formerly named TNF- α) and enzymes such as cyclooxygenase-2 (COX-2) or inducible NO synthase (iNOS) (Figure 4).⁷

The mechanisms of these two pathways above emphasize the eligibility of α,β -unsaturated compounds for triggering the thiol-mediated gene expression in the cell, which makes them attractive to be used as anti-inflammatory agents.

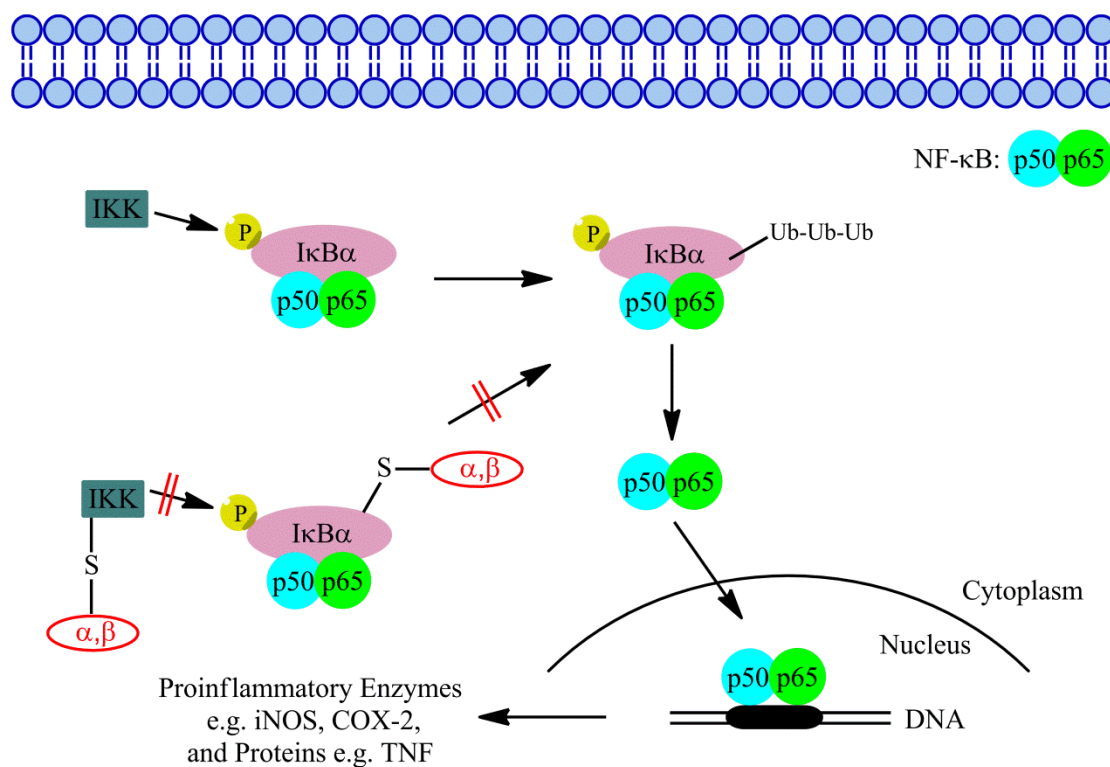


Figure 4: Mechanism of NF-κB inhibition pathway, (NF-κB: nuclear factor-kappa B; a heterodimer of p50 and p65 proteins; IKK: IκB kinase; IκBα: inhibitor of kappa B, α; Ub-Ub-Ub: ubiquitination; α,β: α,β-unsaturated compound; iNOS: inducible NO synthase; COX-2: cyclooxygenase-2; TNF: tumor necrosis factor).

1.2. Chalcones

Chalcones are natural α,β-unsaturated carbonyl compounds from the class of plant polyphenols, which have the structure of 1,3-diphenylprop-2-en-1-ones. They belong to the largest class of plant secondary metabolites and considered to be the biosynthetic precursors of cyclic flavonoids.⁸ Figure 5 shows some plants which are sources of chalcones⁹ and some natural chalcones **2-5** are shown in Figure 6.



Nymphaea caerulea
(Water lily)



Syzygium samarangense
(Wax apple)



Rhus verniciflua
(Varnish tree)



Carthamus tinctorius
(Safflower)



Humulus lupulus
(Hop)



Semecarpus anacardium



Piper methysticum
(Kava)

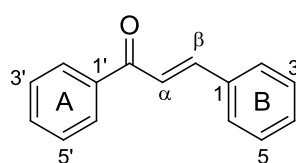


Angelica keiskei
(Ashitaba)

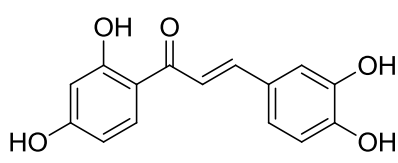


Caesalpinia sappan
(Sappanwood)

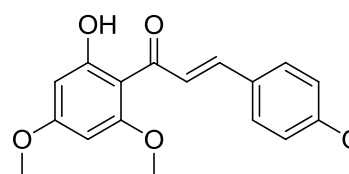
Figure 5: Some sources of chalcones.



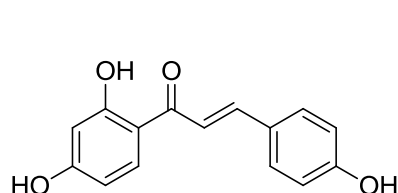
Chalcone (1)



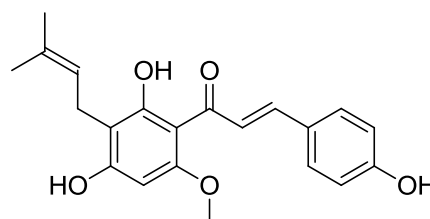
Butein (2)



Flavokawain A (3)



Isoliquiritigenin (4)



Xanthohumol (5)

Figure 6: Chalcone (1) and some examples of natural chalcones (2-5).

The numbering system of chalcones is shown on the structure of chalcone (**1**) (Figure 6). The C=C in the α,β -unsaturated moiety of chalcones can adopt a *Z* (*cis*) or an *E* (*trans*) configuration. Thermodynamically, the *E*-isomer is more stable and all the isolated chalcones are in this form.¹⁰ The *E/Z* photoisomerization of chalcones is known and different isomers may have different biological activity.¹¹ Orientation of the C=O and the C $_{\alpha}$ =C $_{\beta}$ around the single bond is also important in chalcones. They adopt either the *s-cis* or the *s-trans* conformation (Figure 7A). The X-ray crystal structures of many α -unsubstituted chalcones clearly show the preference for the *s-cis* conformer (as in Figure 6), while some α -substituted chalcones have been reported to adopt the *s-trans* conformation,¹² such as the α -Me¹² and α -F¹³ chalcones (Figure 7B).

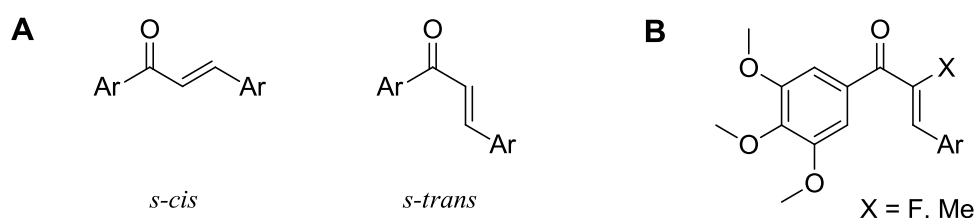


Figure 7: A) *s-cis* and *s-trans* Conformations of chalcones, B) Examples of *s-trans* chalcones. (Examples of *s-cis* chalcones are shown in Figure 6).

1.2.1. Biological activity of chalcones

Chalcones are found in fruits, vegetables, spices and tea. As chemoprotective and chemopreventive agents, chalcones have been reported to possess many biological activities such as anti-inflammatory, antioxidative, antimitotic, antibacterial, antifungal, antimalarial and antileishmanial.^{8-10,14-17} Cytotoxic and antiviral properties were also found.^{8,10,15} As electrophiles, the activity of chalcones is mostly based on the Michael acceptor activity of their α,β -unsaturated carbonyl system as well as their radical scavenging or reductive potential (antioxidative behavior).²

1.2.2. Fine tuning the Michael acceptor activity of chalcones

Despite the ability of Michael acceptors to address certain cysteine residues, they are a neglected class of compounds in drug development.⁸ This is due to possible unspecific reactions of the very strong electrophiles with less reactive thiol groups. Additionally, such electrophiles can be trapped by the cellular glutathione (GSH), whose cysteine residue has only moderate reactivity, and thus leads to reduced activity of potent electrophiles.

Consequently, fine tuning of the Michael acceptor activity of α,β -unsaturated compounds is highly needed to improve specificity.

Some reported studies to assess the reactivity of α,β -unsaturated systems

The assessment of the reactivity of compounds would be a very helpful tool to potentially predict their biological activity. Different approaches have been reported to compare similar natural products and synthetic molecules in order to correlate their biological activity with the reactivity of their α,β -unsaturated carbonyl system, such as NMR spectroscopic analyses and in silico methods. The ^{13}C NMR shift of the β -carbons in a series of drug-like molecules (Figure 8) was used for example to estimate their electrophilicity, then to correlate it with their Michael acceptor activity, reduction potential and photoisomerization.¹⁸

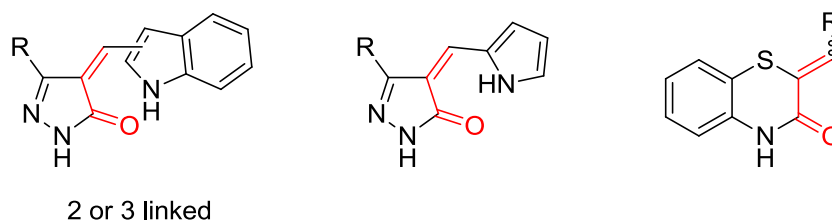


Figure 8: Scaffolds of the drug-like compounds that were used to correlate their electrophilicity (which was estimated using ^{13}C NMR shifts of the β -carbons) with their biological activity.¹⁸

A study on prostaglandins (Figure 9) showed that the calculated LUMO coefficients and net atom charges are in agreement with the proposed 1,4-addition reactivities depicted by their ^{13}C NMR studies.¹⁹

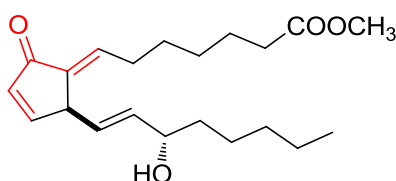


Figure 9: An example of prostaglandins.

Reduction ability of the $\text{C}=\text{O}$ of the chalcones can also be used as an indicator for the electrophilicity of the β -position because of the delocalization of electrons along the α,β -unsaturated moiety.¹⁰ Dimmock and coworkers for example found a good correlation between the reduction potential of the $\text{C}=\text{O}$ functionality and the cytotoxicity of the some chalcones (Figure 10). The higher the reduction potential the more toxic was the compound.²⁰

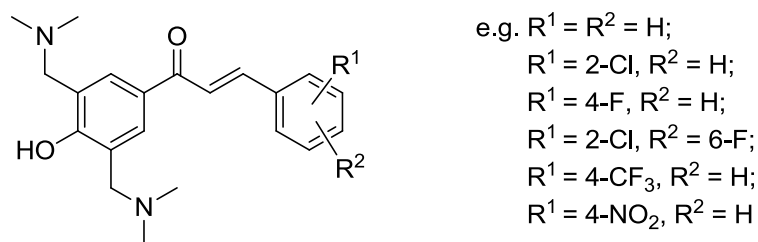


Figure 10: Examples for chalcones investigated by Dimmock.²⁰

Zoete published an interesting study on 30 plant phenylpropenoids and synthetic analogues (Figure 11) that showed a correlation between their E_{HOMO} values and their biological activity as inducers for NAD(P)H:quinone oxidoreductase-1 (NQO1). It was demonstrated that the lower absolute E_{HOMO} value of a compound *i.e.* the lower its reduction potential, the stronger is its electron-donating nature and the greater is its inducer potency.²¹

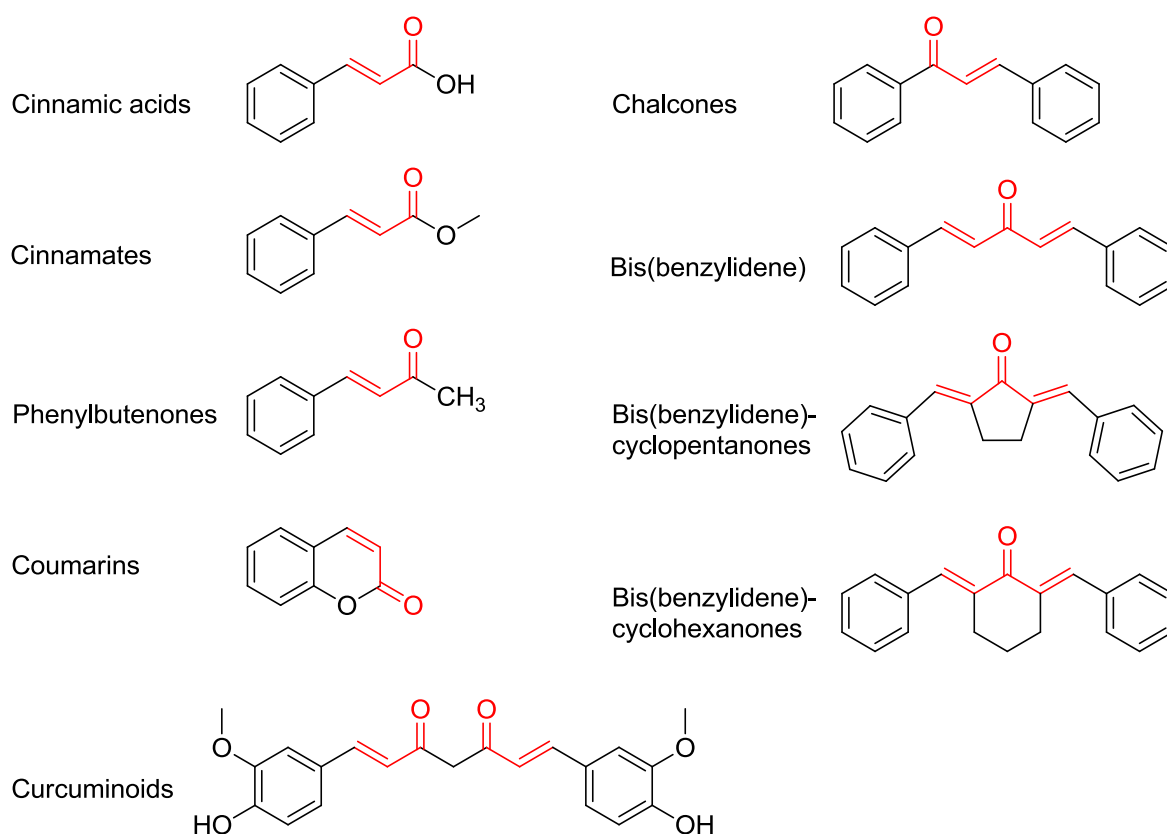


Figure 11: Classes of the plant phenylpropenoids and synthetic analogues that were investigated by Zoete.²¹

The E_{HOMO} and E_{LUMO} energies were also correlated with reduction potential and radical scavenging activity of some synthetic chalcones (Figure 12). The study showed that the

higher E_{HOMO} values, the smaller is the reduction potential. In addition, high E_{HOMO} and E_{LUMO} values lead to high radical scavenging activity.²²

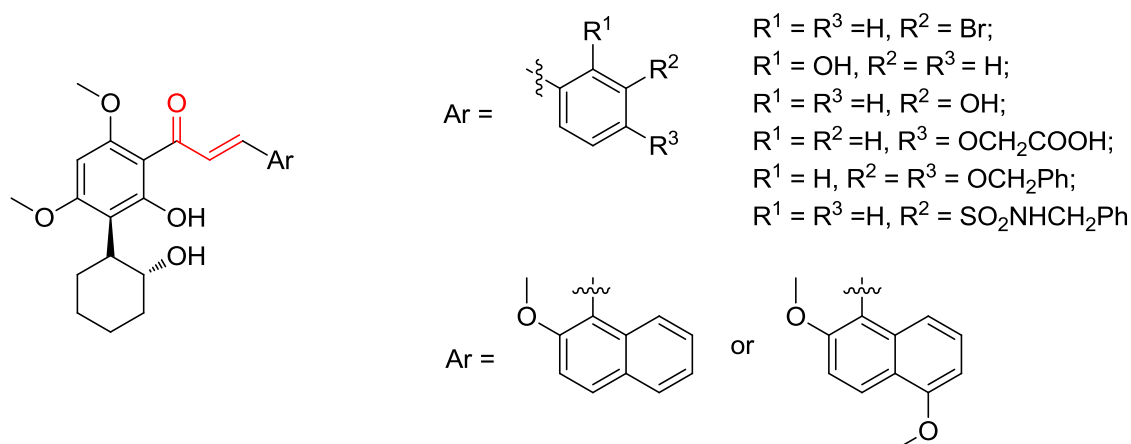


Figure 12: Synthetic chalcones that were investigated for the relationship between their E_{HOMO} and E_{LUMO} energies and their reduction potential and radical scavenging activity.²²

Assays to determine the reactivity of α,β -unsaturated compounds toward thiols

Since the biological activity of Michael acceptors depends on their reaction with cysteine residues of proteins during the thiol-mediated processes, the assessment of their electrophilicity towards thiols in particular is of great interest.

Recently an NMR-based assay was developed and used to group different α,β -unsaturated natural products (e.g. curcumin and (*R*)-carvone) as reversible and irreversible thiol sinks.²³ The ^1H NMR spectra (in DMSO-d_6) were obtained for the compounds in presence of a thiol to identify the thiol-trapping agents among the tested compounds at first, then the reversibility of the reactions was investigated upon dilution of the DMSO-samples with CDCl_3 . This assay is valuable and helps to get important structural information especially when more than one reactive site had reacted in one molecule. But it is not a quantitative assay that can be used to compare a library of compounds with small structural differences like chalcones.

Dinkova-Kostova and Talalay²⁴ determined the rate of the reaction of some α,β -unsaturated compounds with thiols in a 1:1 mixture of MeCN and 100 mM TRIS-HCl pH 7.4 at 25 °C. A good correlation was found between the reactivity of the tested compounds and their ability to elevate cellular Glutathione (GSH).

A facile spectrophotometric kinetic assay was developed during this PhD work to assess the reactivity of the synthesized chalcones toward thiols especially that the conditions of

Dinkova-Kostova and Talalay were not suitable for the stability and the wide range of reactivity of the synthesized chalcones as will be shown later. Additionally the developed assay is based on 96-well plate format which enables fast and simple testing.

Modulating the reactivity of chalcones

The fine tuning of the reactivity of compounds is a promising approach to optimize potent compounds for a specific biological target. A recent example where electrophilicity tuning strategy was successful is the modulation of the reactivity of the CN group in a series of aryl nitriles by variation of the aryl moiety (Figure 13). A good correlation was found between the calculated electrophilicity and the inhibitory activity of the synthesized compounds toward a cysteine protease, *i.e.* higher electrophilicity led to more inhibition. Additionally, it was found that the reduced electrophilicity resulted in reduced cytotoxicity.²⁵

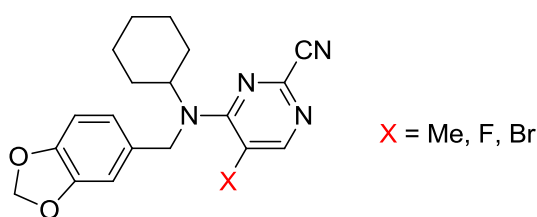


Figure 13: Examples of aryl nitriles whose electrophilicity was correlated with their inhibitory activity towards a cysteine protease.²⁵

In order to modulate the reactivity of chalcones, the strategy of changing the substitution pattern of the aromatic rings of chalcones has been widely used. An example is the 10 fold increase in reactivity of chalcone (**1**, Figure 6) towards sulfur ylids by introducing electron-withdrawing substituents in the B-ring.²⁶ Another strategy which is not that common in literature is to modify the α -position of the α,β -unsaturated carbonyl system. This is a promising concept since it should have a direct and straightforward influence on the reactivity. In 1978 Yamamura investigated the reaction of several α -X-chalcone derivatives (Figure 14) with benzene and acetic acid in the presence of palladium(II)-acetate. He found that when X is a bulky and powerfully electron-withdrawing group, such as CPh, NO₂, COOEt and COOH, the addition of benzene to the carbon-carbon double bond occurs.²⁷ This means that electron-withdrawing groups lead to an enhancement of the electrophilicity of chalcones and therefore the Michael addition takes place easily. This example of the influence of α -substitution on chemical reactivity shows that a great difference in reactivity of chalcones can be achieved by only a single modification.

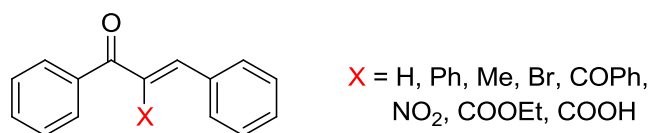


Figure 14: α -X-Chalcones investigated by Yamamura.²⁷

Examples of the influence of α -modification on biological activity are also present. Lawrence *et al.* described the effect of different α -X-substituents ($X = F, CN, COOEt$) and different substitution patterns on the B-ring of a series of chalcones on their biological activity (Figure 15A) exhibiting antimitotic properties caused by inhibition of tubulin polymerization. The most potent compound in their series was the one with $X = F$ and with 3-hydroxy-4-methoxy B-ring.¹³ In a review of antimitotic chalcones and related compounds as inhibitors of tubulin assembly, Ducki pointed out that the enhanced bioactivity of some α -substituted chalcones as antimitotics is attributed to their *s-trans* conformation which increases their affinity for tubulin.²⁸ In another example, the effect of α -X-substituents ($X = CN, COOH, COOMe, OH, OMe, Cl, Br$) in oleanane (Figure 15B) and ursane triterpenoids was shown on inhibition of NO production in mouse macrophages and found that the CN substituent gave the best activity. No correlation was found in this case between the Taft's σ^* values of the α -X-substituents and the biological activity which means that the activity does not depend on the strength of the electron-withdrawing effect of the substituents in this case.²⁹

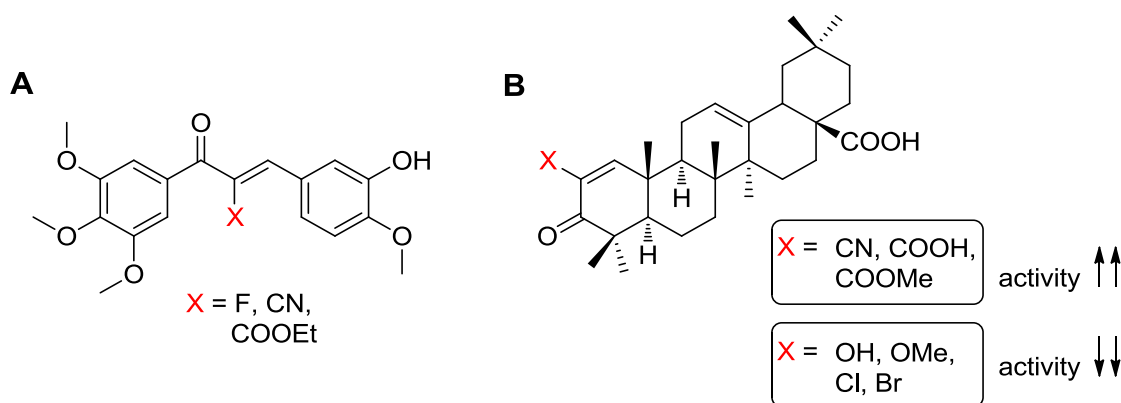


Figure 15: Literature examples of α -X-enones to modulate biological activity: **A)** chalcones as antimitotic agents,¹³ **B)** oleanane triterpenoids as inhibitors of NO production.²⁹

1.2.3. Chalcones as warhead groups – an outlook

‘Warhead group’ is an expression termed for a reactive group that binds covalently to its target. It is the major element of an activity-based probe (ABP) which consists of three

elements (Figure 16): 1) a warhead group, 2) a tag that allows detection of the covalent enzyme-probe complex and 3) a linker (spacer) that joins the two previous elements together.³⁰ ABPs are used to profile the functional state of enzymes in complex proteomes in a strategy which is called activity-based protein profiling (ABPP). This strategy is also a powerful tool to characterize the selectivity of enzyme inhibitor on a global scale.³¹

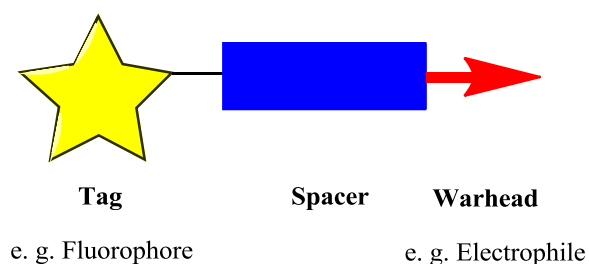


Figure 16: Schematic representation of an activity-based probe (ABP).

As reactive electrophiles, Michael acceptors including chalcones may be utilized as warhead groups due to their ability to bind covalently to cysteine residues of proteins. Chalcones may serve a useful extension of the toolbox of ABPs to be used in labeling studies to identify reactive cysteines of the proteins for example. This would be invaluable, since there are many families of enzymes that use cysteine-dependent chemical transformation such as proteases.³² Recently, a proteome-wide study applied an ABP that has an electrophilic iodoacetamide probe ended with an alkyne (Figure 17). This ABP was used to label cysteine residues in proteins then click-chemistry strategy was used to add a tag to the ABP. After digestion, the released probe-labeled peptides were analyzed by LC-MS/MS to identify the modified cysteines. This approach was also used to distinguish between cysteines of heightened reactivity and less reactive cysteines and thus indicating the importance of hyper-reactive cysteines. Additionally, it was envisioned that by a variation of the electrophile reactivity one will be able to target different subsets of cysteines.³² Since the electrophilic activity of chalcones can be modulated, they may be utilized in the same manner, especially that there is a strong need for tailored electrophiles to trigger the thiol-mediated gene expression and to develop new warhead groups.³⁰ In another study, chalcones proved to block active-site cysteines in cysteine proteases such as cathepsin B and L or topoisomerase I, and by that display anticancer properties.³³

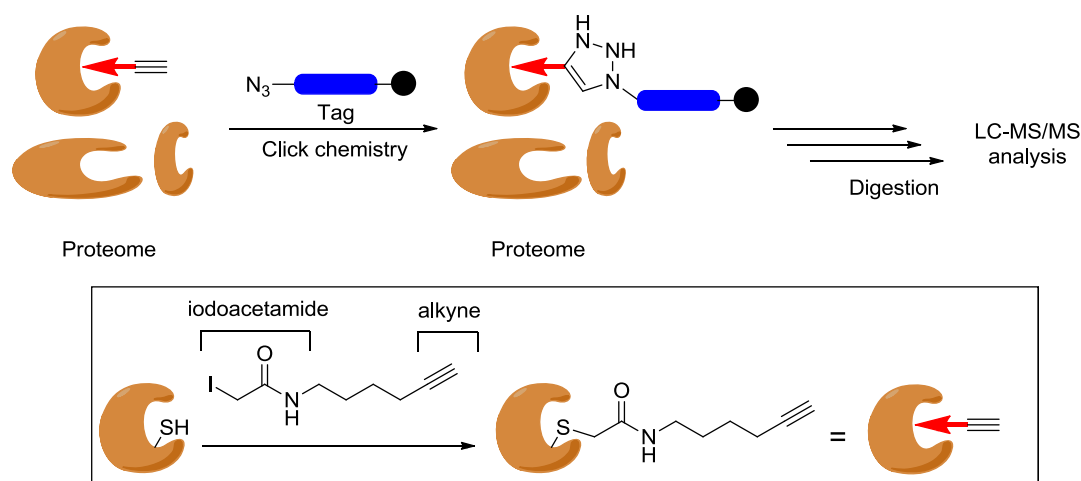


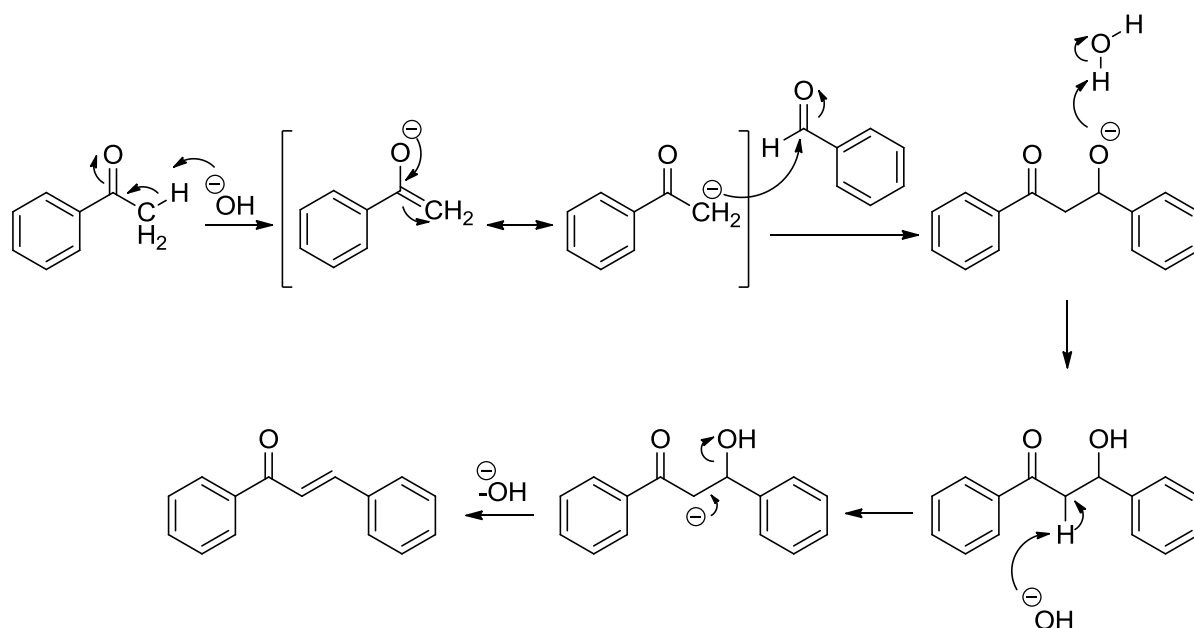
Figure 17: The approach of labeling cysteine residues of proteins using ABP and click chemistry.

Consequently, fine-tuning the electrophilicity of chalcones could potentially lead to more potent irreversible covalent inhibitors that can attach to recognition motifs with high selectivity. If so, this can unfold the positive effects of covalent irreversible drugs such as a high degree/complete inactivation of the target, potentially reduced dosing, a higher efficiency, or circumvention of some resistance pathways.³¹

1.2.4. Synthetic methods for the preparation of chalcones

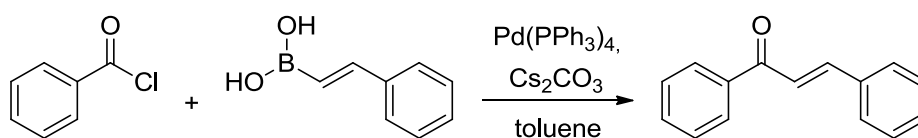
1.2.4.1. α -Unsubstituted chalcones

Many methods have been described for the preparation of chalcones. Nevertheless, the most common strategy to synthesize them is the base-mediated Claisen-Schmidt condensation of an aldehyde and a ketone in a polar solvent such as MeOH.¹⁰ Variable bases were used to achieve this condensation such as NaOH and KOH. Scheme 1 below shows the mechanism of this reaction. The acetophenone in presence of a base forms the enolate that can attack the aldehyde; once the alkoxide is formed the dehydration occurs to furnish the chalcone. Bukhari³⁴ reported recently in a mini review some known methods and various catalysts used for the synthesis of chalcones, among these catalysts are LiOH·H₂O, anhydrous Na₂CO₃, BiCl₃ and NaOH-Al₂O₃.



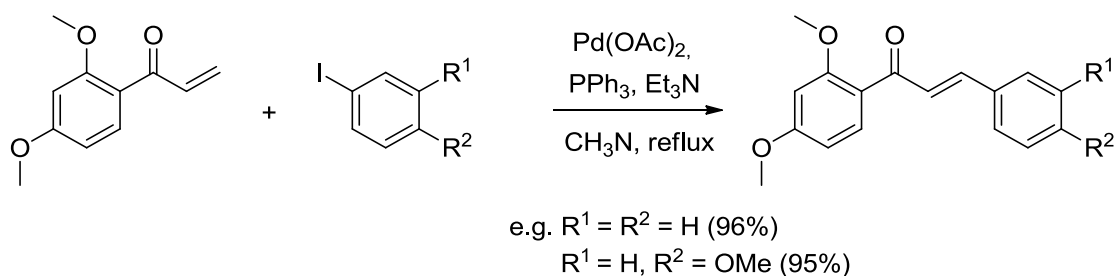
Scheme 1: Base-mediated Claisen-Schmidt condensation mechanism.

Synthesis of chalcones by Suzuki coupling between benzoyl chlorides and phenylvinylboronic acids as in Scheme 2 is another reported method to get the chalcones.³⁵



Scheme 2: Chalcone synthesis via Suzuki coupling.³⁵

Heck coupling reaction between aryl vinyl ketones and aryl iodides (Scheme 3) was also reported as a method for the synthesis of chalcones in good to excellent yields.³⁶



Scheme 3: Chalcone synthesis via Heck coupling.³⁶

To reduce reaction time and facilitate work-up, ultrasound³⁷ and microwave irradiation³⁸⁻³⁹ were also investigated for the synthesis of chalcones.

1.2.4.2. α -Substituted chalcones

Substitutions on the α -position of the α,β -unsaturated carbonyl unit have been achieved in synthetic triterpenoids (Figure 15B),²⁹ mimetics of those (Figure 18A),⁴⁰ chalcones (Figure 14, 15A)^{13,27} and the 3(2*H*)-furanones (Figure 18B)⁴¹ which were synthesized by Simon Lindner in our group.

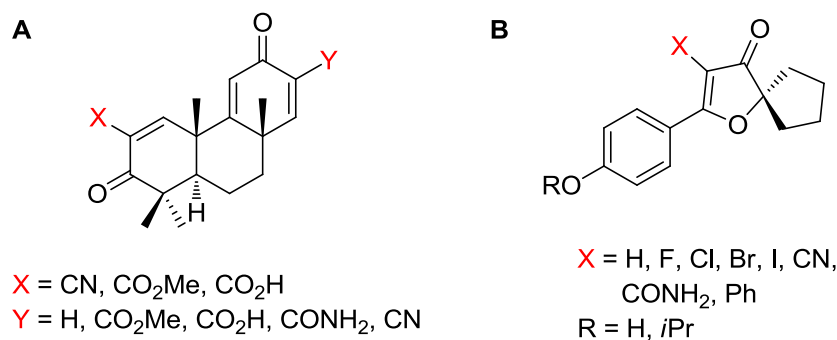
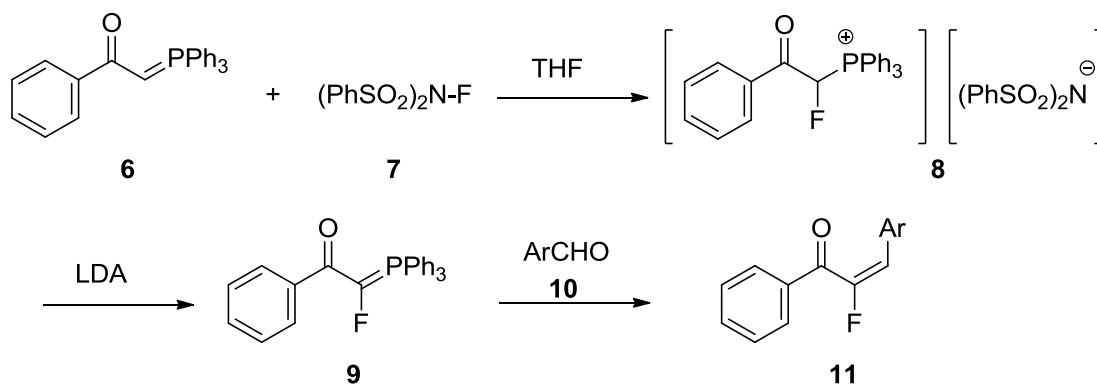


Figure 18: Examples of α -substituted enones.

Some reported methods for the synthesis of α -X-chalcones are discussed below.

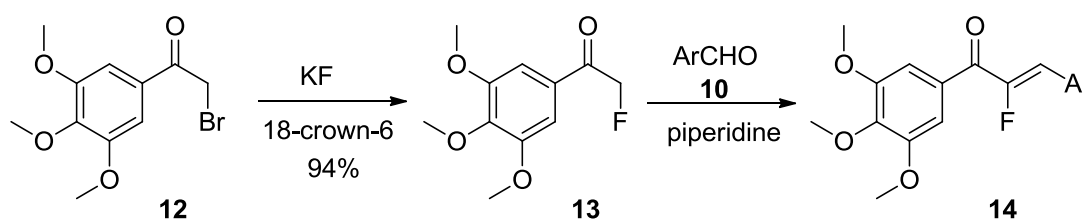
α -Halogenated chalcones (α -F/Cl/Br/I-chalcones)

Huang⁴² reported a one-pot stereoselective method for the preparation of (*E*)- α -F-chalcones by Wittig reaction via the corresponding α -F-substituted ylids (Scheme 4). Ylid **6** was allowed to react with equimolar amounts of *N*-fluorodiphenylsulfonamide (**7**) to form salt **8**, and then a strong base (lithium diisopropylamine, LDA) was added to form ylid **9** smoothly. The α -F-ylid **9** can then react *in situ* with an aldehyde **10** to give (*E*)- α -F-chalcones **11**.



Scheme 4: Synthesis of (*E*)- α -F-chalcones by Wittig reaction.⁴²

A series of (Z)- α -F-chalcones **14** were prepared by Lawrence and co-workers¹³ by aldol condensation between the α -F-acetophenone **13**, that was synthesized from the corresponding α -Br-acetophenone **12**, and an aldehyde **10** using piperidine as a base (Scheme 5). They claimed that they used a catalytic amount of piperidine, but 3-5 drops of piperidine is not a catalytic amount with respect to 0.5 mmol of the ketone and equimolar amounts of the aldehyde they used. Their compounds showed potent cytotoxic and tubulin inhibitory properties.



Scheme 5: Synthesis of (Z)- α -F-chalcones by Lawrence.¹³

As antimitotic agents, Edwards *et al.*⁴³ prepared some (Z)- α -Cl-chalcones (Figure 19) by chlorination of the α -H-chalcones using sulfuryl chloride (SO_2Cl_2) but with low yields.

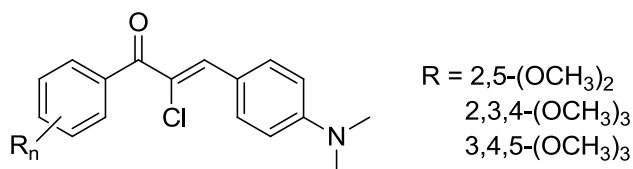
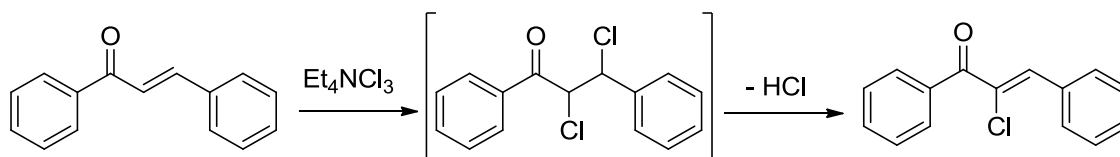


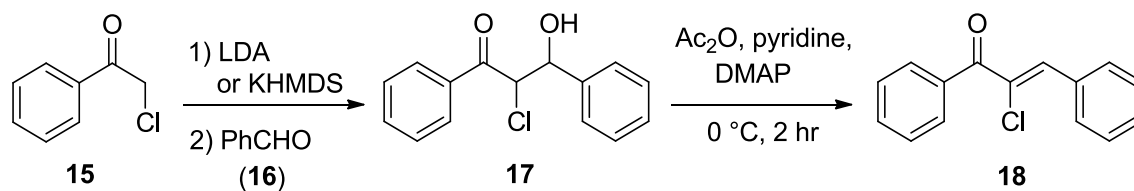
Figure 19: (Z)- α -Cl-Chalcones prepared by Edwards.⁴³

Mioskowski group⁴⁴ showed that the chlorination of α,β -unsaturated ketones can be achieved by Et_4NCl_3 , suggesting that it may occur via vicinal α,β -dichloroketones which undergo spontaneous dehydrohalogenation (Scheme 6).



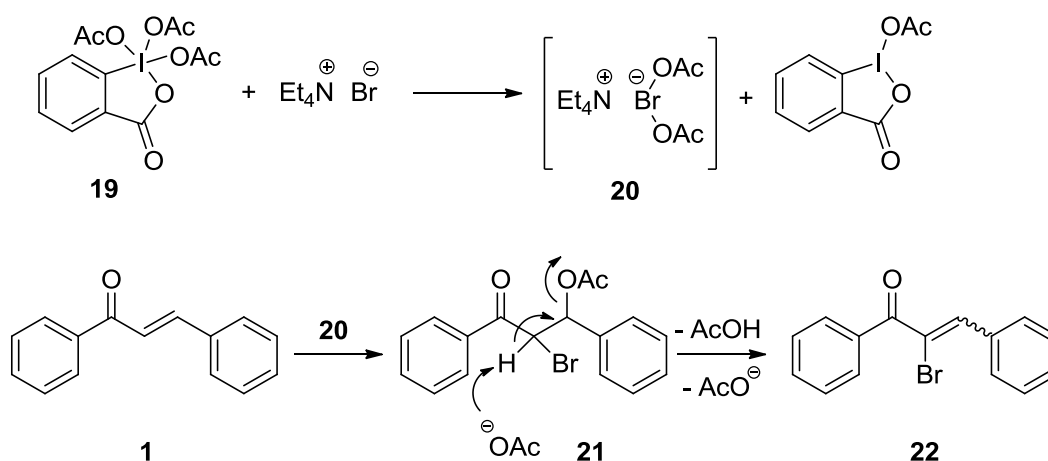
Scheme 6: Synthesis of α -Cl-chalcone using Et_4NCl_3 .⁴⁴

In 2002, Concellón⁴⁵ reported the preparation of (Z)- α -chloro- α,β -unsaturated ketones by reacting α -chloro- β -hydroxyketones with acetic anhydride, pyridine and 4-dimethylaminopyridine (DMAP) in high yields. An example is shown in Scheme 7. The α -Cl-acetophenone **15** was treated with LDA or potassium hexamethyldisilazide (KHMDs) to get the enolate that was reacted with benzaldehyde **16** to get the α -chloro- β -hydroxyketone **17**. Elimination of water from **17** gave α -Cl-chalcone **18**.



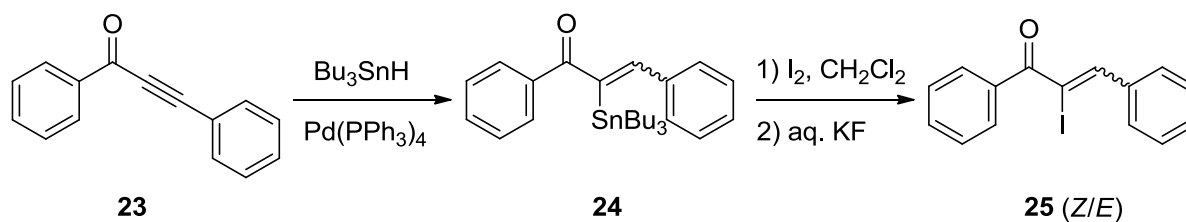
Scheme 7: The synthesis of (Z)- α -Cl-chalcone (**18**) by Concellón.⁴⁵

The classical bromination method of chalcones with Br_2 affording α,β -dibromochalcones which can undergo dehydrobromination in presence of a base (e.g. Et_3N) to get the α -bromochalcones is widely used in literature.⁴⁶⁻⁴⁸ Akamanchi *et al.*⁴⁹ in 2002 published a novel one-step procedure for the preparation of α -bromo- α,β -unsaturated carbonyl compounds from the corresponding α,β -unsaturated carbonyl compounds utilizing Et_4NBr as a brominating agent in presence of Dess-Martin periodinane (DMP **19**, Scheme 8). When they applied their method on chalcone (**1**) they got a mixture of a (Z/E)- α -bromo-chalcone but Z was the major isomer. Scheme 8 shows the mechanism of bromination by their method. Two acetate ligands of DMP (**19**) firstly transfer to Et_4NBr forming tetraethylammonium[di(acyloxy)bromate (I)] (**20**) which then adds to the double bond of chalcone (**1**) to form bromoacetoxylated intermediate **21**. The acetate ion formed in the reaction acts as a base and abstracts the α -H furnishing the α -Br-chalcone **22**.



Scheme 8: Mechanism of α -bromination of chalcones using Et_4NBr in presence of DMP (**19**).⁴⁹

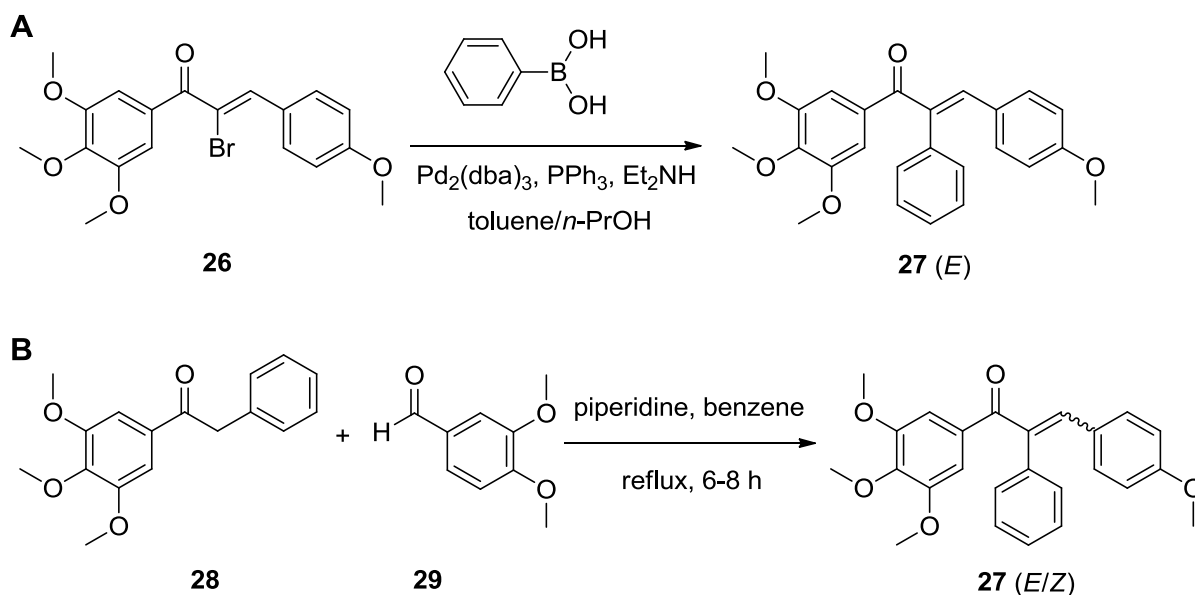
As an example for the synthesis of α -I-chalcone, Rossi⁵⁰ published in 1993 a Pd-catalyzed method that involves a reaction between 1-alkynyl ketone **23** and Bu_3SnH followed by iododestannylation of **24** to afford a *Z/E* mixture of α -I-chalcone **25** (Scheme 9).



Scheme 9: Pd-Catalyzed synthesis of α -I-chalcone **25** by Rossi.⁵⁰

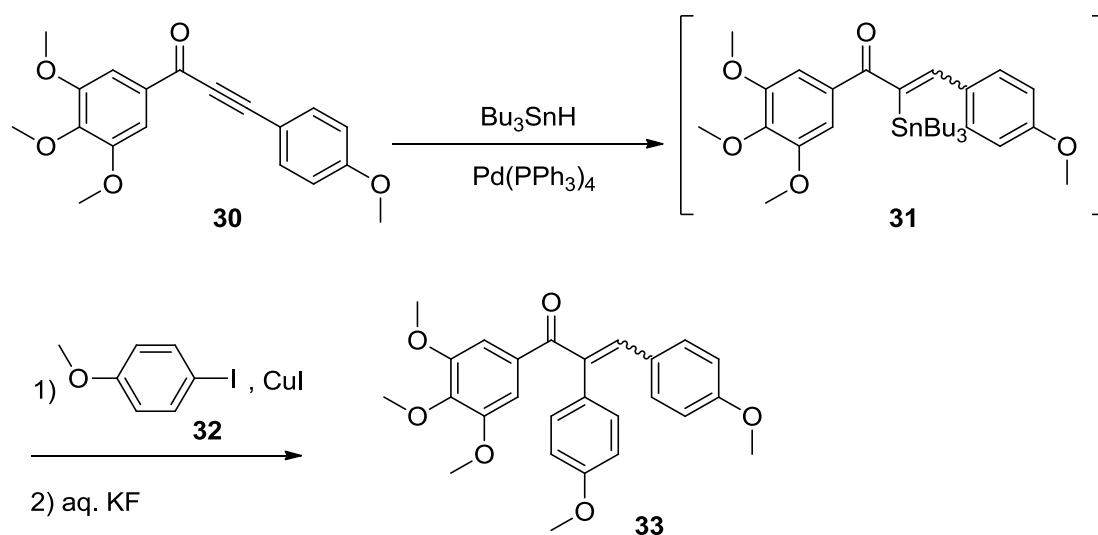
α -Aryl-chalcones

Regarding the α -aryl-chalcones (e.g. **27**), they can be prepared by Suzuki coupling the way Ducki and coworkers⁴⁸ did it for example (Scheme 10A). They started with the α -Br-chalcones (e.g. **26**) and could get moderate to good overall yields (26–84%) with high selectivity for the *E*-isomer in all of their α -aryl-chalcones prepared by this method. In the same article, Ducki showed another method to prepare α -aryl-chalcones (Scheme 10B) by aldol condensations of a range of substituted benzaldehydes (e.g. **29**) with ketones (e.g. **28**) catalyzed by piperidine as a base in good to excellent yields (58–97%). The products from these reactions were isolated as *E/Z* mixtures. Ducki *et al.* evaluated their α -aryl-chalcones for their ability to inhibit tubulin assembly.



Scheme 10: Methods used by Ducki⁴⁸ for the synthesis of α -aryl-chalcones, **A**) by Suzuki coupling, **B**) by aldol condensation.

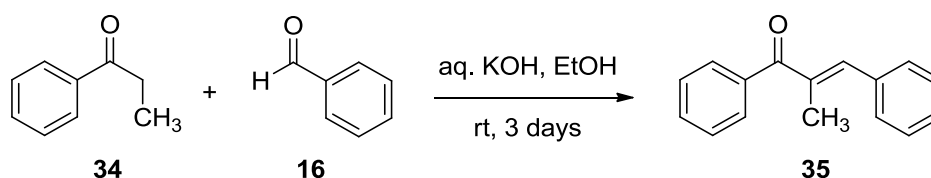
Flynn *et al.*⁵¹ used a one-pot Pd-mediated hydrostannylation/cross coupling protocol to get the α -aryl-chalcones (e.g. **33**) as shown in the following example in Scheme 11. Hydrostannylation of alkyne **30** gives compound **31** that upon Cu-catalyzed cross coupling with an organic halide **32** furnishes the α -aryl-chalcone **33**. They got initially the *Z*-isomer, but they reported its isomerization to the thermodynamic mixture of *E*- and *Z*- isomers.



Scheme 11: Pd-Mediated hydrostannylation/cross coupling protocol to get α -aryl-chalcones.⁵¹

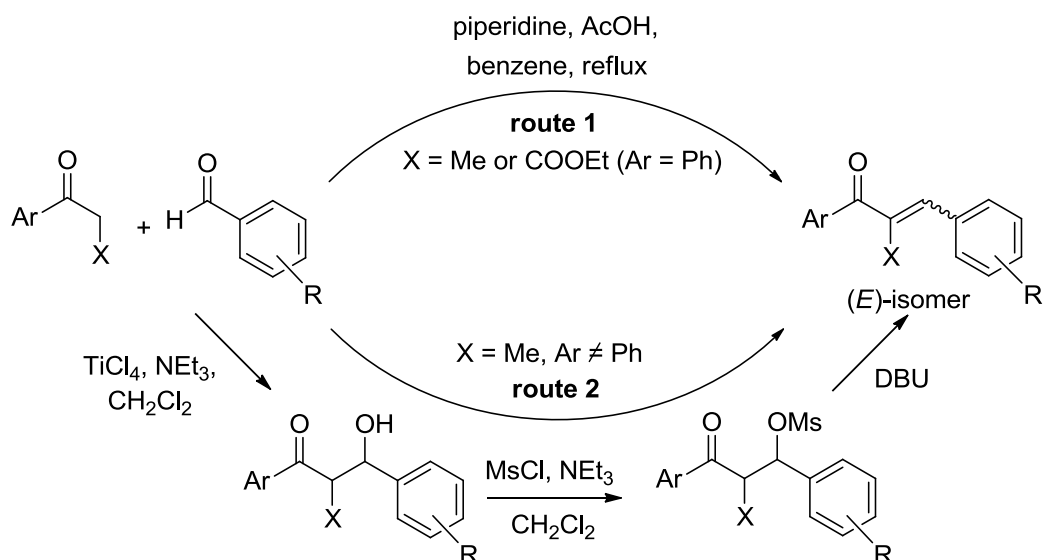
α -Me/COOEt/COOH/CN chalcones

As antimitotic agents, Edwards⁴³ prepared (*E*)- α -Me-chalcones by the piperidinium acetate mediated aldol condensation of propiophenones and benzaldehydes using EtOH as a solvent. In 2008, Bolm⁵² prepared the (*E*)- α -Me-chalcone **35** (Scheme 12) by condensation of propiophenone **34** with benzaldehyde (**16**) using aq. KOH as a base in EtOH.



Scheme 12: Preparation of (*E*)- α -Me-chalcone **35** by Bolm.⁵²

Batey *et al.*⁵³ prepared (*E*)- α -Me-chalcones and (*E*)-COOEt-chalcones using Edwards reagents (piperidine and glacial AcOH)⁴³ but benzene was used as a solvent instead of EtOH and it was heated to reflux using a Dean Stark apparatus (route 1, Scheme 13). For the synthesis of (*E*)- α -Me-chalcones where the A-ring is a substituted aromatic ring (not just a phenyl) a three-step aldol protocol was used (route 2, Scheme 13). The TiCl₄-mediated aldol reaction between the aldehyde and an appropriately substituted propiophenone afforded *syn*- β -hydroxy ketones that were then immediately converted to the corresponding mesylates, which underwent elimination giving the (*E*)- α -Me-chalcones.

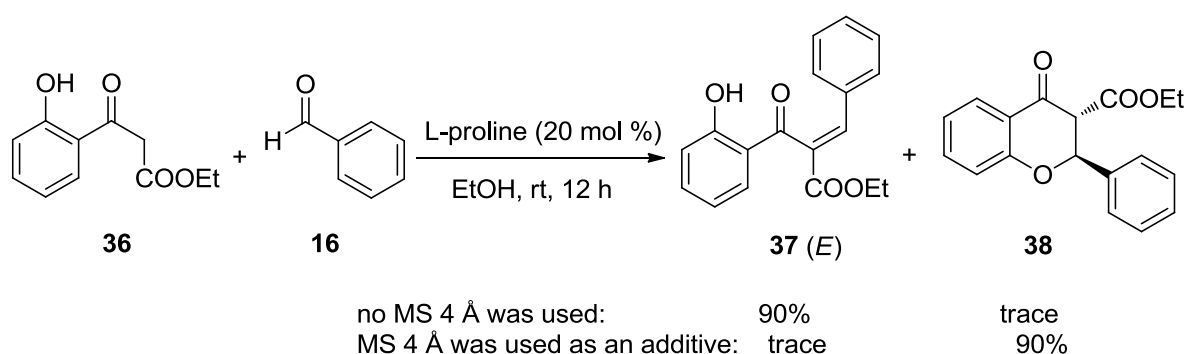


Examples: route 1: (X = COOEt) : R = 4-NO₂; 3-NO₂; 2-NO₂; 4-Cl; 3-Cl; 2-Cl;
4-Me; 3-Me; 4-OMe; 3-OMe; 2-OMe; 4-CN; 3-CN
(X = Me) : R = 3-CN; 3-Cl

route 2: (X = Me) : Ar = 4-Cl-C₆H₄, R = 4-NO₂;
Ar = 4-OMe-C₆H₄, R = 4-Cl

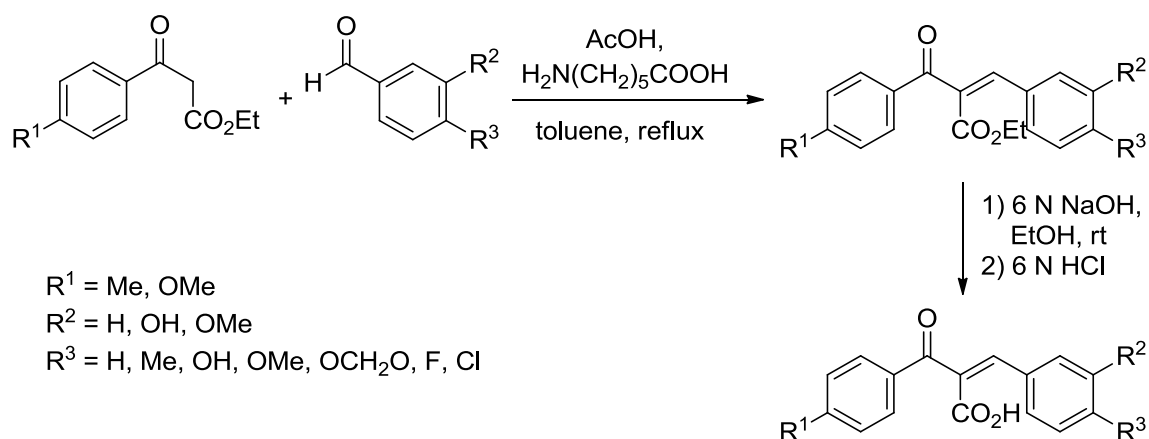
Scheme 13: Synthesis of (*E*)-α-Me-chalcones and (*E*)-COOEt-chalcones by Batey.⁵³

The Knoevenagel condensation reaction of ethyl 3-(2-hydroxyphenyl)-3-oxopropanoate (**36**) and benzaldehyde (**16**) was investigated by Zhu *et al.* in 2009 (Scheme 14).⁵⁴ They found that a catalytic amount of L-proline was enough to catalyze the reaction giving the desired product (α-COOEt-chalcone **37**) in 90% yield under very mild reaction conditions. But, when they performed the reaction in the presence of molecular sieves 4 Å (MS 4 Å) or with piperidine or pyrrolidine as a catalyst, only the *trans* cyclized product (flavanone) **38** was obtained. Moreover, they found that the α-COOEt-2'-OH-chalcone **37** could be easily converted to the cyclized isomer **38** under basic conditions.



Scheme 14: Synthesis of (*E*)-α-COOEt-chalcone **37** by Zhu.⁵⁴

Wang *et al.*⁵⁵ synthesized some α -COOH-chalcones as potential aldose reductase inhibitors. They firstly prepared the corresponding α -COOEt-chalcones via Knoevenagel condensation of a series of ethyl benzoylacetates with a range of benzaldehydes in the presence of acetic acid in toluene and using 6-aminohexanoic acid as a catalyst. The produced α -COOEt-chalcones were hydrolyzed with 6 N NaOH in EtOH and subsequently acidified with 6 N HCl to afford the (*Z*)- α -COOH-chalcones (Scheme 15). They claimed to have the *Z*-isomer for both their α -COOEt- and α -COOH-chalcones, but it was not mentioned how the configuration of the compounds was determined. On the other hand, Batey *et al.*⁵³ determined the stereochemistry of their α -COOEt-chalcones (Scheme 13) by the chemical shift of the vinylic protons, saying that the vinylic protons of the (*E*)- α -COOEt-chalcones resonate further downfield ($\delta = 8.2$ -7.8 ppm) compared to the corresponding (*Z*)-isomers ($\delta = 7.2$ -6.8 ppm). Applying this rule on Wang compounds indicates that their assignment of the configuration might not be correct, since the shift of the β -H for their α -COOH-chalcones is 7.59-7.85 ppm.



Scheme 15: Synthesis of α -COOH-chalcones by Wang with assignment of configuration as shown.⁵⁵

Based on the $^3J_{\text{C-H}}$ coupling constants of $\text{C}_{\text{ketone-H}}$ and $\text{C}_{\text{ester-H}}$, the correct stereochemistry of α -COOEt-chalcones can be determined unambiguously. This method was used by Deng⁵⁶ based on Kingsbury article⁵⁷ in assigning the *E*-configuration for his α -COOEt-chalcones which have $^3J_{\text{C-H}}$ of 9.8-9.9 Hz for their $\text{C}_{\text{ketone-H}}$ and 7.6-7.7 Hz for their $\text{C}_{\text{ester-H}}$. Kingsbury reported that in the *Z*-isomer the $^3J_{\text{C-H}}$ of the $\text{C}_{\text{ester-H}}$ is larger than that of the $\text{C}_{\text{ketone-H}}$, while the vice versa is true for the *E*-isomer as indicated in Figure 20.

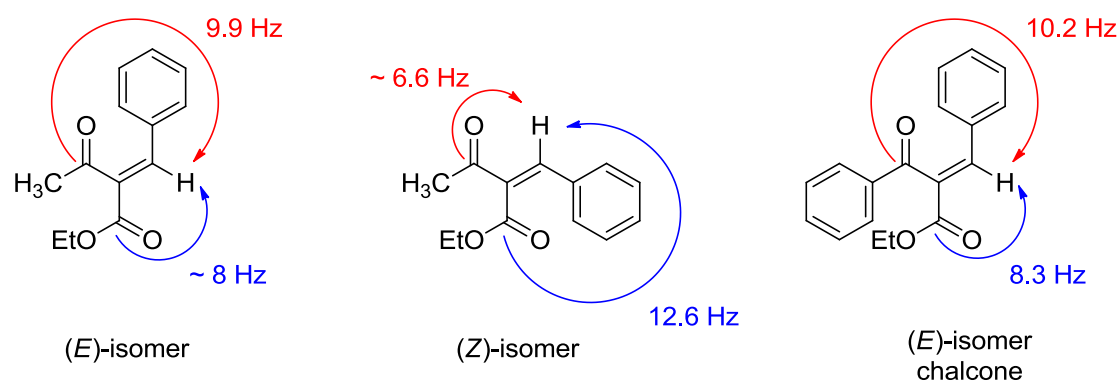
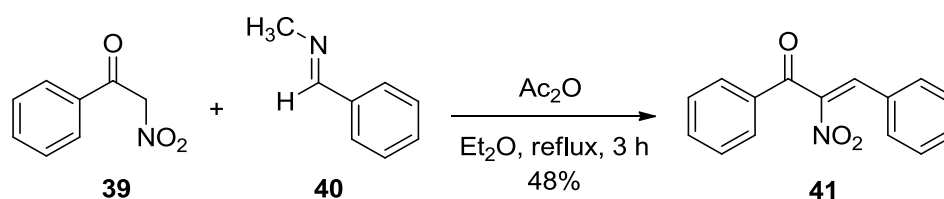


Figure 20: $^3J_{C-H}$ Coupling constants of $C_{\text{ketone}}\text{-H}$ and $C_{\text{ester}}\text{-H}$ of (E)- α -COOEt- and (Z)- α -COOEt- α,β -unsaturated carbonyl compounds found by Kingsbury.⁵⁷

Lawrence¹³ reported the synthesis of α -COOEt and α -CN-chalcones by Knoevenagel condensation of benzaldehydes with the β -ketoesters or β -ketonitriles, respectively. Piperidine was used as a base and the reactions were conducted in EtOH just in the same way the α -F-chalcones was prepared (Scheme 5).

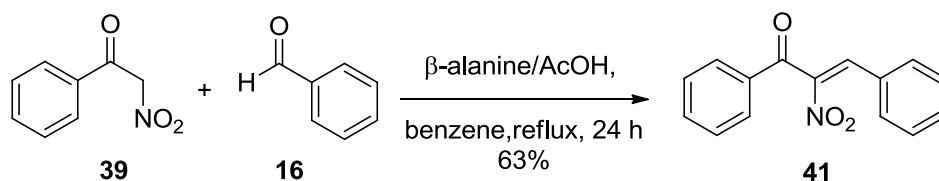
α -NO₂-chalcones

Concerning the α -NO₂-chalcones, in 1955 Dornow⁵⁸ prepared α -NO₂-chalcone **41** (Scheme 16) by reacting 1.0 eq. of α -NO₂-acetophenone **39** with equimolar amount of the Schiff base **40** that was generated from benzaldehyde (**16**) before. The reaction was done in Et₂O in presence of 1.1 eq. acetic anhydride (Ac₂O).



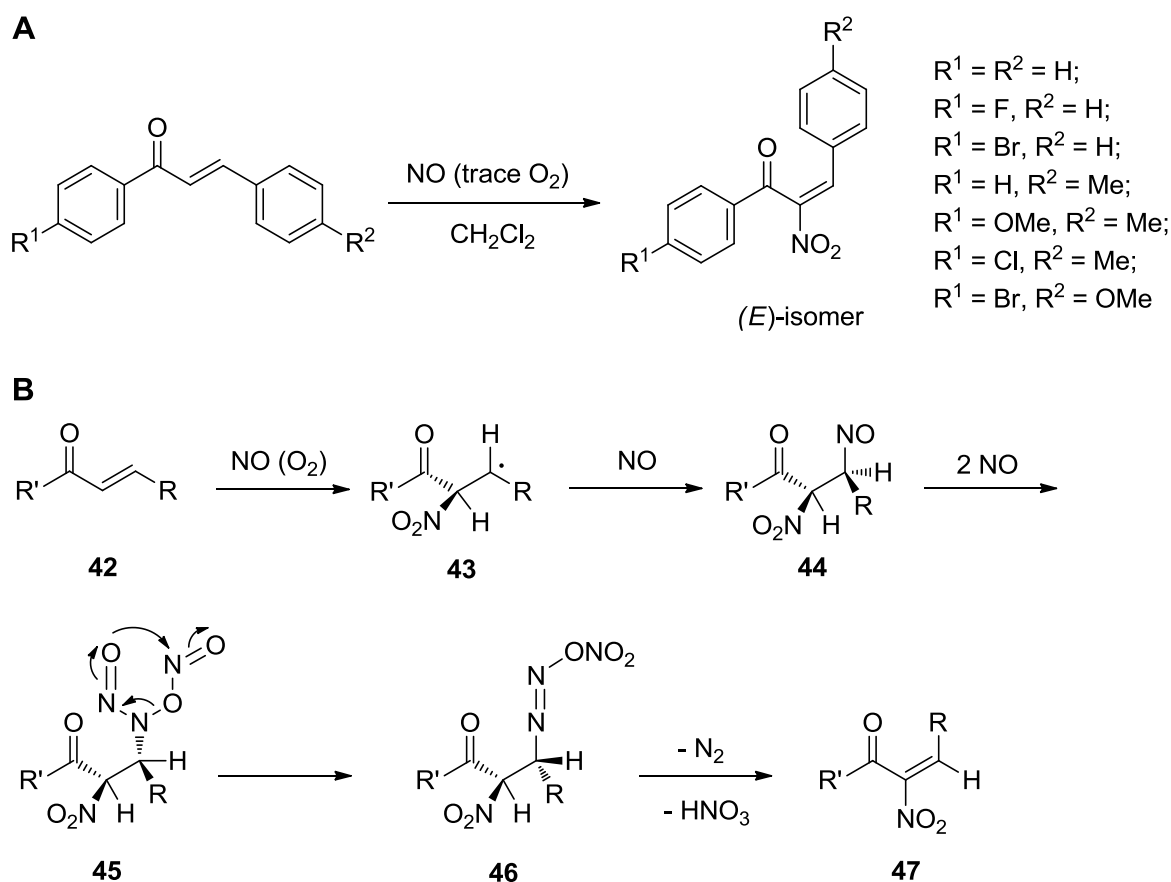
Scheme 16: Synthesis of α -NO₂-chalcone **41** by Dornow.⁵⁸

In 2002 Sagitullina⁵⁹ prepared the same α -NO₂-chalcone **41** by reacting 1.0 eq. of α -NO₂-acetophenone **39** with 1.6 eq. benzaldehyde (**16**) in presence of 0.084 eq. β -alanine and 2.8 eq. glacial acetic acid (Scheme 17). The reaction was done in benzene that was heated at reflux using a Dean Stark apparatus.



Scheme 17: Synthesis of α -NO₂-chalcone **41** by Sagitullina.⁵⁹

A regio- and stereoselective nitration method of chalcone derivatives was published in 2006 by Wu *et al.*⁶⁰ The method is based on using nitric oxide to get (*E*)- α -NO₂-chalcones exclusively in good yields (Scheme 18A).



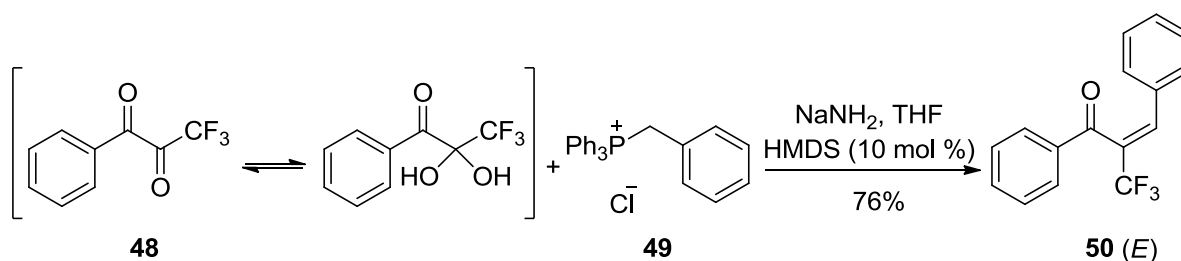
Scheme 18: A) Synthesis of α -NO₂-chalcones by Wu, B) Mechanism of nitration of α -H-chalcones with NO.⁶⁰

The authors claimed that first a trace of oxygen oxidizes NO to NO₂, and then electrophilic addition of NO₂ to the α -position of the α -H-chalcone **42** gives a carbon-centered radical **43**. Coupling of NO with the β -carbon of **43** affords the nitroso compound **44** that upon addition of another 2 eq. of NO gives *N*-nitroso-*N*-nitrite **45**. Rearrangement of **45** furnishes a diazonium nitrate **46** which undergoes anti-elimination affording the α -NO₂-chalcone **47** as

an *E*-isomer which is much more stable than the *Z*-isomer (Scheme 18B). Only a trace amount of O₂ is needed at the beginning to form NO₂ that initiates the reaction, any further needed NO₂ is produced from the reaction of NO with the eliminated HNO₃. It was found that an excess of NO₂ leads to many side reactions.⁶⁰

α -CF₃-chalcones

As an α -CF₃-chalcone, the only reported example which is not substituted on the β -position was published in 2013 by Cahard⁶¹ who prepared (*E*)- α -CF₃-chalcone **50** by Wittig olefination of **48** with ylid **49** as shown in Scheme 19.



Scheme 19: Synthesis of (*E*)- α -CF₃-chalcone **50** by Cahard.⁶¹

1.3. Determination of the anti-inflammatory activity

As a prerequisite for other biological tests, the determination of cell viability or cytotoxicity in presence of the tested compounds is normally performed so that the subsequent biological tests can be done at a suitable concentration. These assays are also used to study the effect of the tested compounds on cell viability or to show direct cytotoxic effects that lead to cell death, which is very important in drug development. Hence the MTT cell viability assay was performed for most of the synthesized chalcones in this study. Then the influence of these chalcones on the production of NO was measured by the Griess assay as an indication for their potential anti-inflammatory activity. A description of the theory behind these two biological assays is given below.

1.3.1. Cell viability

There are a variety of assay methods that can be used to determine the cell viability. These assays can be divided into two main categories: assays that measure cell death and assays that quantify biochemical processes which are considered as viability markers. Some known methods to assess the viability are presented here.⁶²

1- Vital dyes: Vital dyes are fluorescent or colored molecules that can be used to discriminate between living and dead cells. Some of them work by marking the dead ones and the others by marking the living ones. Cytofluorometry (Figure 21) or fluorescence microscopy is then used to count the cells.

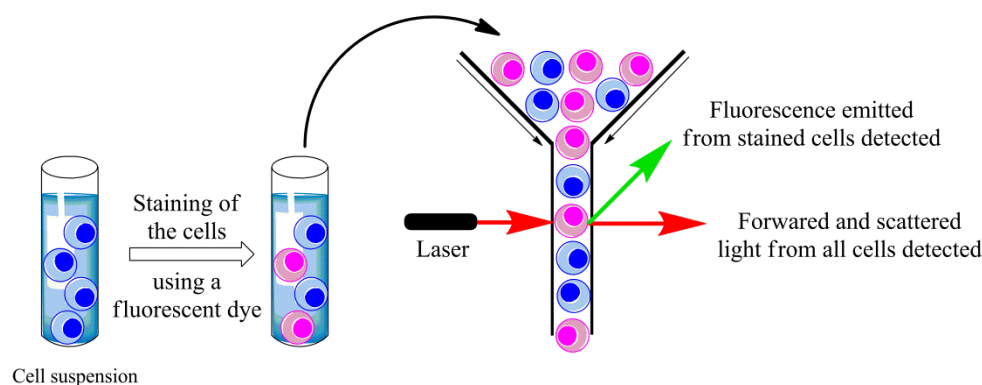


Figure 21: The principle of cytofluorometry.

2- Extracellular release of proteins: Measuring the release of intracellular proteins into cell culture supernatants is a way to quantify the rupture of the plasma membrane of cells. Fluorometric or colorimetric measurement of lactate dehydrogenase (LDH) is an example.

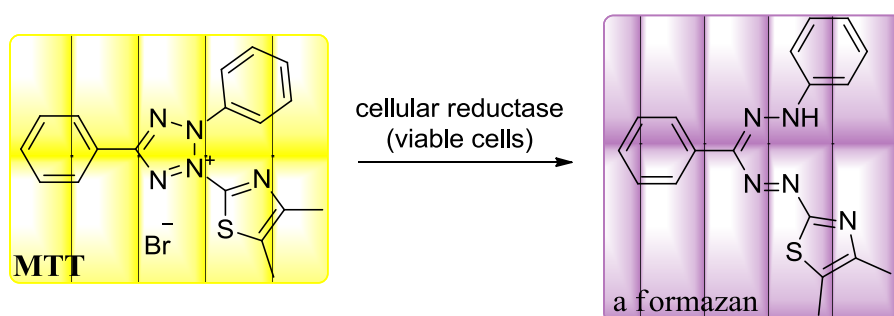
3- Cellular metabolism: Many biomarkers of cells can be used to measure their viability. ATP which is essential for cellular life is an example. Quantification of the amount of intracellular ATP can be used as an indication of cell viability, since it is produced in living cells. The well-known MTT test is another example of this category. It depends on the ability of cellular mitochondrial reductases to convert MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) into a violet-colored compound (Scheme 20) that can be easily quantified spectrophotometrically.

4- Cell attachment: Normally, the viable cells tend to adhere to the culture substrate while the injured cells round up and detach with time, which is concurrent with cell death. Hence, the degree of the attachment of the cells can be used to reflect their viability.

MTT assay

The MTT assay is a sensitive and quantitative colorimetric assay that is used to measure the viability of the cells. In this assay, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) which is dissolved in a physiologically balanced solution is added to cells in culture and incubated for a specific time. During the incubation time the viable cells

reduce the yellow water-soluble MTT using mitochondrial dehydrogenase enzymes to a formazan dye which is a purple and water-insoluble compound (Scheme 20). The formazan is solubilized using SDS (sodium dodecyl sulfate), for example, then the intensity of its purple color is determined spectrophotometrically. Since only viable cells have active metabolism, the amount of formazan produced is directly proportional to the number of viable cells.⁶³



Scheme 20: Conversion of the yellow MTT to a violet formazan dye during the MTT assay.

1.3.2. The anti-inflammatory activity through inhibition of iNOS (NO assay)

Nitric oxide (NO) is an important physiological messenger and regulator molecule in diverse biological systems. It has important roles in neurotransmission in central nervous system and in peripheral nerves for example. Additionally, high levels of NO are produced in response to inflammation and mediate proinflammatory and destructive effects. NO has many biological effects depending on its concentration and place of production. Some of these effects are direct which are mediated by NO itself. The other effects are indirect and they are mediated by reactive nitrogen species that are produced by the reaction of NO with superoxide anion (O_2^-) or with oxygen (O_2) (Figure 22). So the molecular mechanism mediating the biological activities of NO can be classified into three groups: 1) NO reacts readily with transition metals (e.g. Fe, Cu, Zn) which are present in the catalytic sites of enzymes, and by that reaction NO regulates the activity of many enzymes, 2) the NO^+ which is produced upon reaction of NO with O_2 is able to induce S-nitrosylation of cysteine residues of proteins and thus leads to modification of the activity of those proteins and 3) the peroxynitrite ($ONOO^-$) that is produced when NO reacts with superoxide anion (O_2^-) is a nitrating agent and a strong oxidizing agent which is able to modify proteins, lipids and nucleic acids.⁶⁴

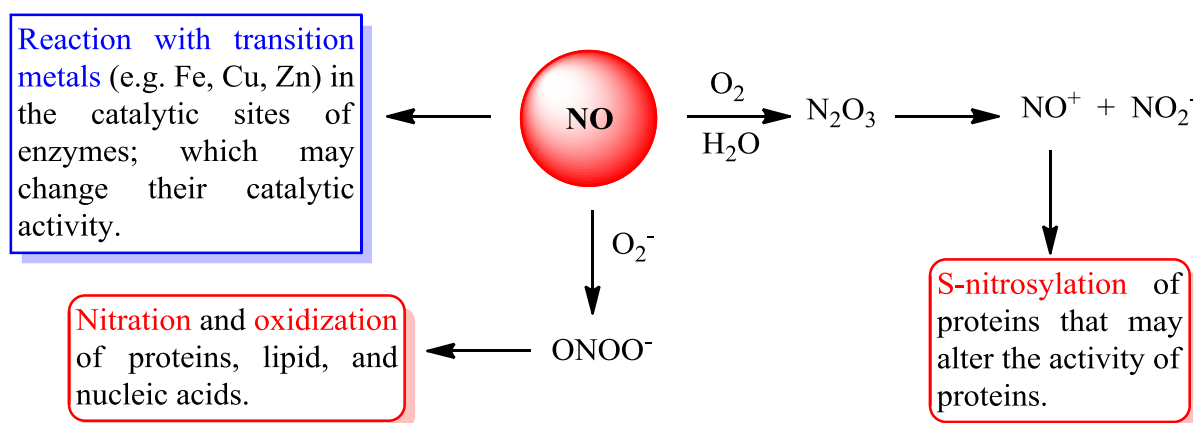
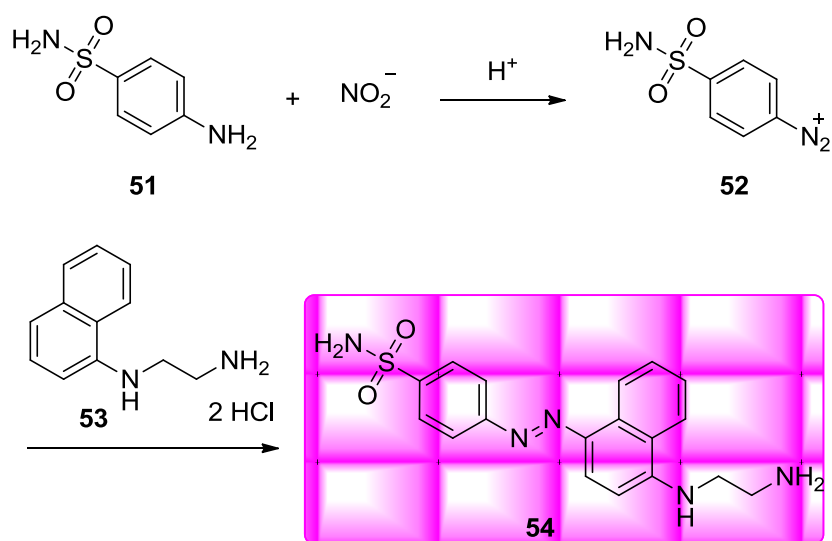


Figure 22: Molecular mechanisms that mediate the biological activities of NO.

NO synthesis is catalyzed by nitric oxide synthase (NOS) enzymes. Three different isoforms of these enzymes have been characterized, the neuronal NOS (nNOS), endothelial NOS (eNOS) and the inducible NOS (iNOS). The first two are found in resting cells and are activated by high intracellular calcium concentration. In contrast, iNOS is not found in resting cells and is produced in many inflammatory and tissue cells only as a result of stimulation. In humans, iNOS is produced upon inhibition of the NF- κ B pathway (Figure 4). Proinflammatory cytokines such as tumor necrosis factor (TNF) and microbial products such as lipopolysaccharide (LPS) are examples of stimulants of iNOS gene expression. High levels of NO are produced as a result of inflammatory stimuli and mediate proinflammatory and destructive effects.⁶⁴ Therefore, the inhibition of NO production is a way to demonstrate anti-inflammatory activity and the quantification of the amount of NO produced by the proinflammatory protein iNOS is a valuable tool to study the anti-inflammatory activity of compounds.

Generally, NO can be quantified by many methods such as Griess assay, chemiluminescence, and electrochemistry. Using chemiluminescence, the detection of NO involves its reaction with ozone forming activated NO_2 (NO_2^*) that upon relaxation to NO_2 emits a photon which can be detected by a photomultiplier tube. The analytical signal is proportional to the instantaneous concentration of NO, hence the NO is measured in a direct way.⁶⁵ The detection of NO electrochemically can be achieved either by reduction of NO to $\text{N}_2\text{O}_2^{2-}$ or by its oxidation to NO_3^- . To determine the NO concentration, the current is measured and compared to a calibration curve.⁶⁵ The most widely used method to measure NO is the Griess assay due to its low cost, simple execution and easy data analysis. In the Griess assay, the NO is determined indirectly by measuring the nitrite (NO_2^-) which is a product of NO's

autooxidation.⁶⁵ This assay depends on a diazotization reaction (Scheme 21) that was originally described by Griess. In acidic medium, the NO_2^- is reacting with sulfanilamide (**51**) to form a diazonium salt **52** that gives a pink azo dye **54** upon reacting with *N*-1-naphthylethylenediamine dihydrochloride (**53**). So, upon treating a sample that contains NO_2^- with the Griess reagent, nitrite is detected and analyzed by formation of a pink color that can be monitored spectrophotometrically. The absorbance of the measured sample is then compared to the absorbance of similarly treated NO_2^- standards via a calibration curve. The measured NO_2^- concentration is proportional to the concentration of NO released.⁶⁵



Scheme 21: Chemical reactions involved in the measurement of NO_2^- using the Griess assay.

2. AIM OF THE PRESENT WORK

This work aims to fine tune the chemical reactivity and biological activity of chalcones. Since the electrophilic nature of chalcones is essential for their biological activity which often depends on thiol-mediated regulation processes, the first objective of this work is to develop a facile screening assay to assess the electrophilicity of chalcones in thia-Michael additions. This would be very valuable, since there is no known simple and efficient quantitative method to compare the electrophilicity of different chalcones and other α,β -unsaturated carbonyl compounds. Understanding the Michael acceptor activity of the α,β -unsaturated carbonyl group would help in the rational design of potential drugs.

The second and major aim of this work is to prepare a library of α -modified chalcones and to study the influence of the α -substitution of the α,β -unsaturated carbonyl system on both the chemical reactivity against thiols (thia-Michael addition) and the biological activity concerning the inflammation proteins HO-1 and iNOS.

Finally, to study the effect of a substituent in the α -position of the α,β -unsaturated carbonyl unit of chalcones in presence of a 2'-hydroxy group, the last aim is to synthesize a series of α -X-2'-hydroxychalcones and compare their biological activity with the α -X-2'-OMe analogues.

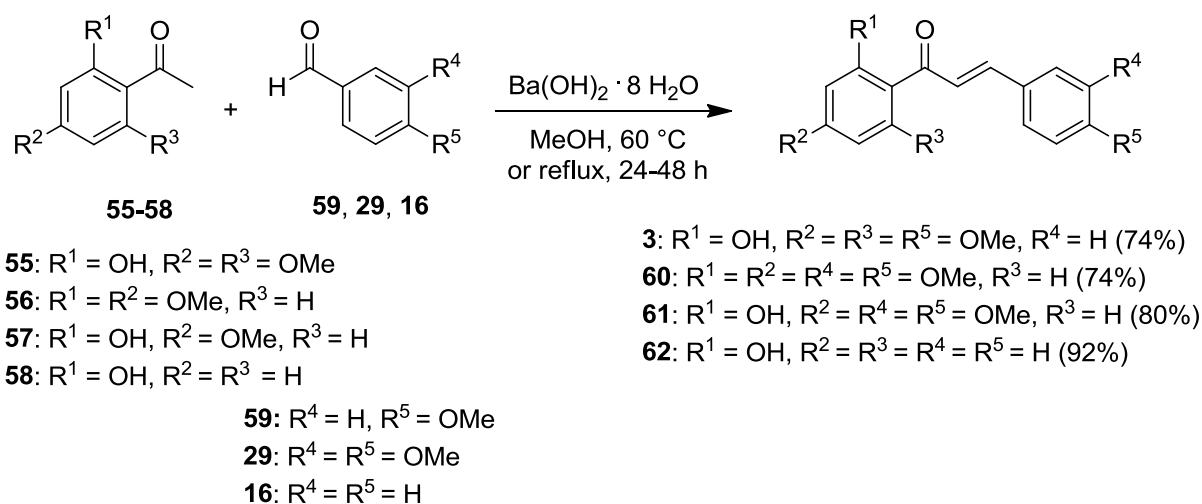
3. RESULTS AND DISCUSSION

The wide range of biological activities of chalcones and the possibility to modulate their reactivity make them attractive targets for research. Aiming to fine-tune the reactivity of chalcones, three different sets of chalcones were synthesized and their activity as potential anti-inflammatory agents was investigated. These are α -H-chalcones with different substitution patterns, α -substituted-tetramethoxychalcones and α -substituted-2'-hydroxy-trimethoxychalcones. The synthetic details are presented first followed by the assessment of chemical reactivity of the synthesized chalcones and finally their anti-inflammatory activity is discussed.

3.1. Synthesis of the chalcones

3.1.1. Synthesis of α -H-chalcones

Four known α -H-chalcones **3**, **60-62** were synthesized applying the classical Claisen-Schmidt condensation of acetophenones **55-58** with benzaldehydes **59**, **29** and **16** using $\text{Ba}(\text{OH})_2 \cdot 8 \text{H}_2\text{O}$ as a base and MeOH as a solvent (Scheme 22).⁶⁶



Scheme 22: Synthesis of α -H-chalcones.

All of the synthesized α -H-chalcones adopt the *E*-configuration. This is proven by the $J_{\text{H}\alpha\text{-H}\beta}$ coupling in their ^1H NMR spectra* which is in the range reported for the *E*-chalcones (15-16 Hz).¹² The X-ray structures of 2',3,4,4'-tetramethoxychalcone (α -H-TMC, **60**)⁶⁷ and its 2'-hydroxy analogue (α -H-HC, **61**) (Figure 23) showed clearly the *E*-configuration and the

* The two doublets can also appear as a pseudo singlet (higher order signal).

s-cis conformation of these two chalcones. Remarkably, the 2'-OH in chalcone **61** leads to a smaller dihedral angle between the two aromatic rings (11.38°) compared to that of chalcone **60** (26.88°), which implies more conjugation in the system in the case of **61**. This effect of the OH at the 2'-position can be rationalized by its intramolecular H-bonding with the carbonyl functionality forming a 6-membered ring.

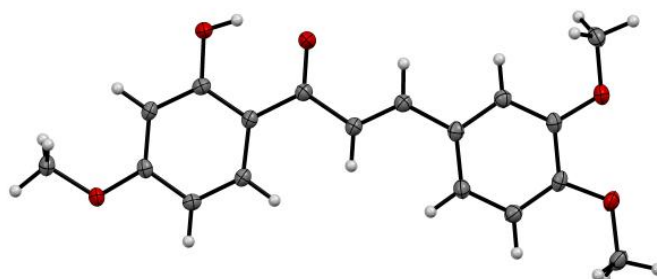
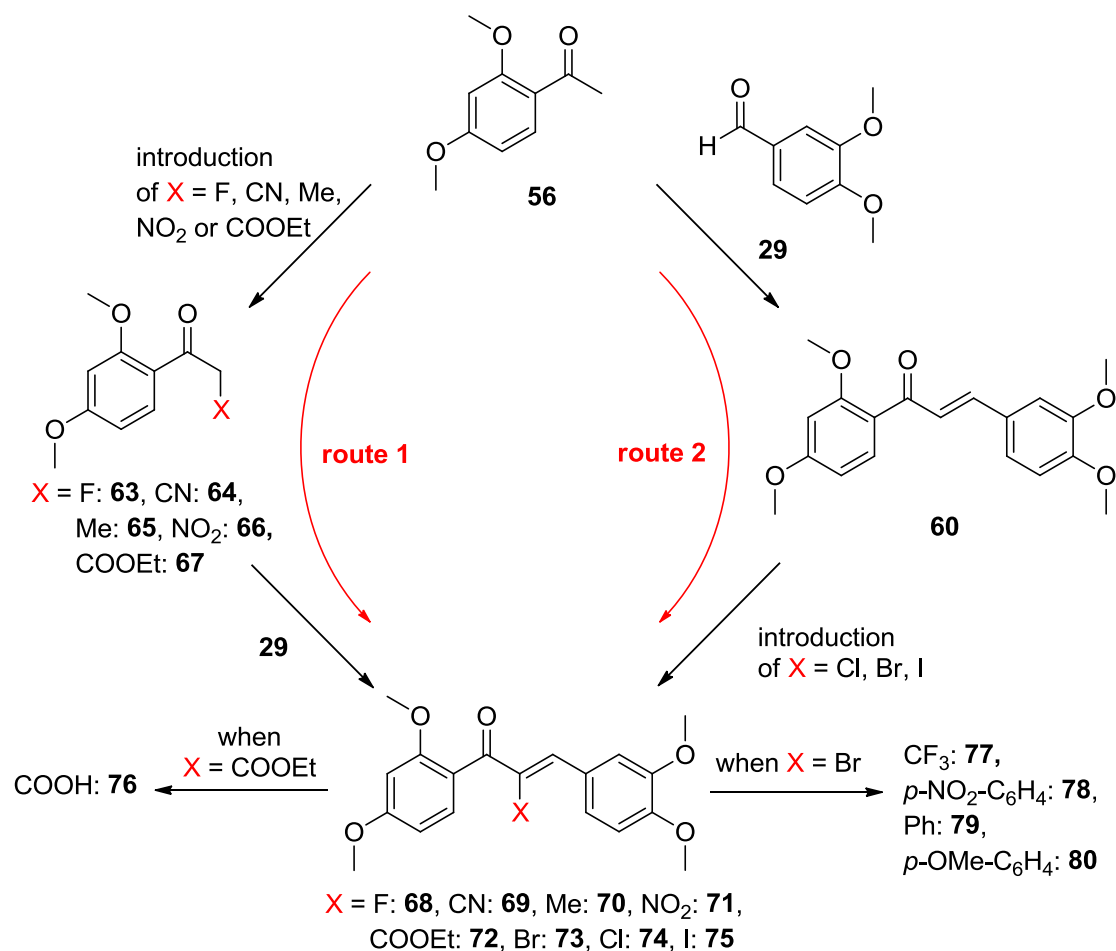


Figure 23: X-Ray structure of 2'-hydroxy-3,4,4'-trimethoxychalcone (α -H-HC, **61**).

3.1.2. Synthesis of α -X-2',3,4,4'-tertramethoxychalcones (α -X-TMCs)

To fine-tune the reactivity of chalcones, the natural-product-like 2',3,4,4'-tetramethoxy-chalcone (α -H-TMC, **60**) was chosen as a scaffold to introduce X-substituents in its α -position. In order to get a potentially broad range of activity, X-substituents with diverse electronic properties were chosen. So, thirteen α -X-derivatives of **60** were prepared, some with electron-withdrawing X-groups like CN, NO₂, CF₃ and halogens which are supposed to enhance the Michael acceptor activity and some others with electron-donating X-substituents such as Me and Ph which are expected to lead to less electrophilicity compared to the parent chalcone **60**.

In order to introduce the α -X-substituent, two synthetic strategies were followed. The first one was to introduce X to acetophenone **56** to get α -X-acetophenones **63-67**, then Claisen-Schmidt condensation with aldehyde **29** was performed (route 1, Scheme 23). Chalcones α -F-TMC (**68**), α -CN-TMC (**69**), α -Me-TMC (**70**), α -NO₂-TMC (**71**) and α -COOEt-TMC (**72**) were prepared from their corresponding acetophenones **63-67** following this route. The second approach which was applied to get α -Br-TMC (**73**), α -Cl-TMC (**74**) and α -I-TMC (**75**) was to introduce X directly in α -H-TMC (**60**) (route 2, Scheme 23). The α -CF₃-TMC (**77**) and the α -aryl-TMCs (**78-80**) were prepared from α -Br-TMC (**73**). While α -COOH-TMC (**76**) was prepared from the ester derivative **72**. Synthetic and structural details are given below.



Scheme 23: The general routes to get the α -X-TMCs.

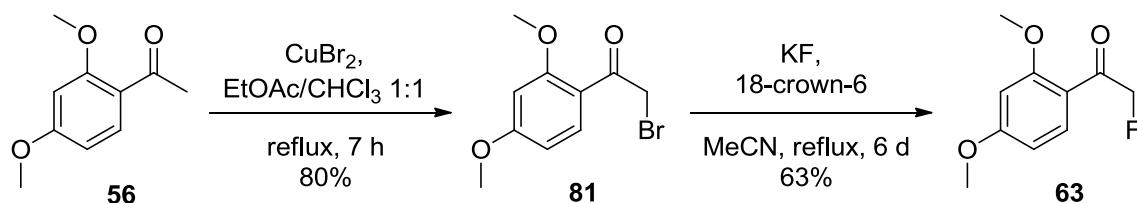
Synthesis of α -F/CN/Me/ NO_2 /COOEt-TMCs (**68-72**)

Chalcones **68-72** were prepared according to route 1 (Scheme 23), so their precursors *i.e.* α -X-acetophenones ($X = F, CN, Me, NO_2, COOEt$) were prepared beforehand. The synthesis of these acetophenones is discussed below followed by the synthesis of the targeted chalcones.

Synthesis of α -X-acetophenones ($X = F$ (**63**), CN (**64**), Me (**65**), NO_2 (**66**), $COOEt$ (**67**)):

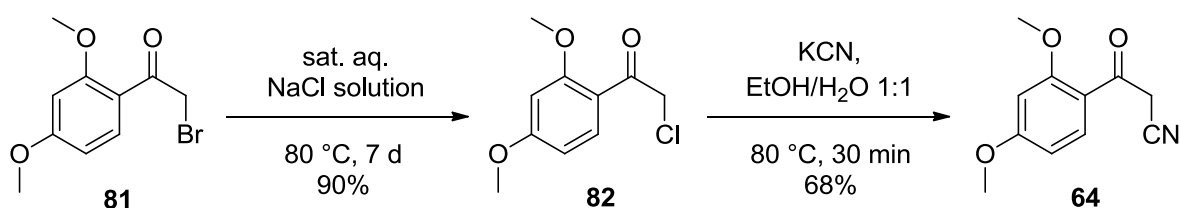
The α -F-acetophenone **63** was prepared in a yield of 63 % by nucleophilic substitution reaction from the corresponding α -Br-acetophenone **81** using KF and 18-crown-6 ether in dry MeCN (Scheme 24).¹³ KF has to be dried very well before the reaction, and the crown ether should be water-free. The crown ether enhances the reaction by complexation with K^+ that leads to activation of the F^- . To insure monobromination, the α -Br-acetophenone **81** was prepared utilizing a high dilution method which was established in our group by Paul Baumeister.⁶⁸ Thus, the bromination of acetophenone **56** which was dissolved in

EtOAc/CHCl₃ (1:1) solvent mixture in a concentration of 4.8 mM was accomplished by portion-wise addition of 2.2 eq. of CuBr₂. The reaction mixture was heated at reflux for 7 h, increasing the reaction time led to dibromination.



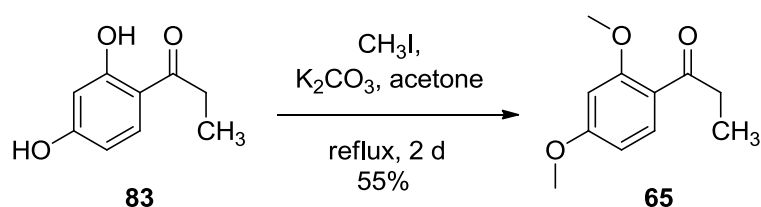
Scheme 24: Synthesis of α -F-acetophenone **63**.

To get the α -CN-acetophenone **64** (Scheme 25), α -Br-acetophenone **81** was stirred in brine affording the 90% of α -Cl-acetophenone **82**⁶⁹ which was transformed into the α -CN analogue **64** using KCN in EtOH/H₂O (1:1) in a 68% yield.⁷⁰ In order to get a higher yield of **64**, this two-step procedure was preferred over the direct conversion of the Br-acetophenone **81** into the CN derivative **64**.



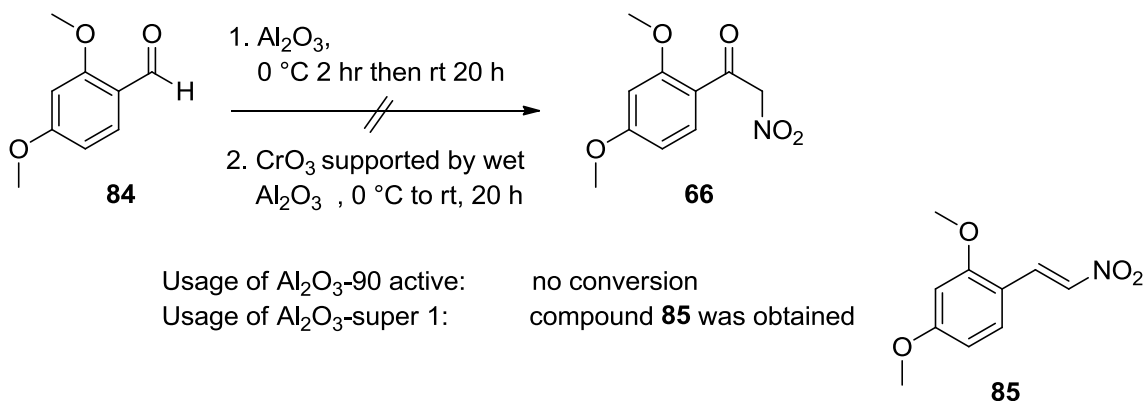
Scheme 25: Synthesis of α -CN-acetophenone **64**.

Methylation of the commercially available 1-(2,4-dihydroxyphenyl)propan-1-one (**83**) using CH₃I and K₂CO₃ in acetone afforded the α -Me-acetophenone **65** with a yield of 55% (Scheme 26).⁷¹



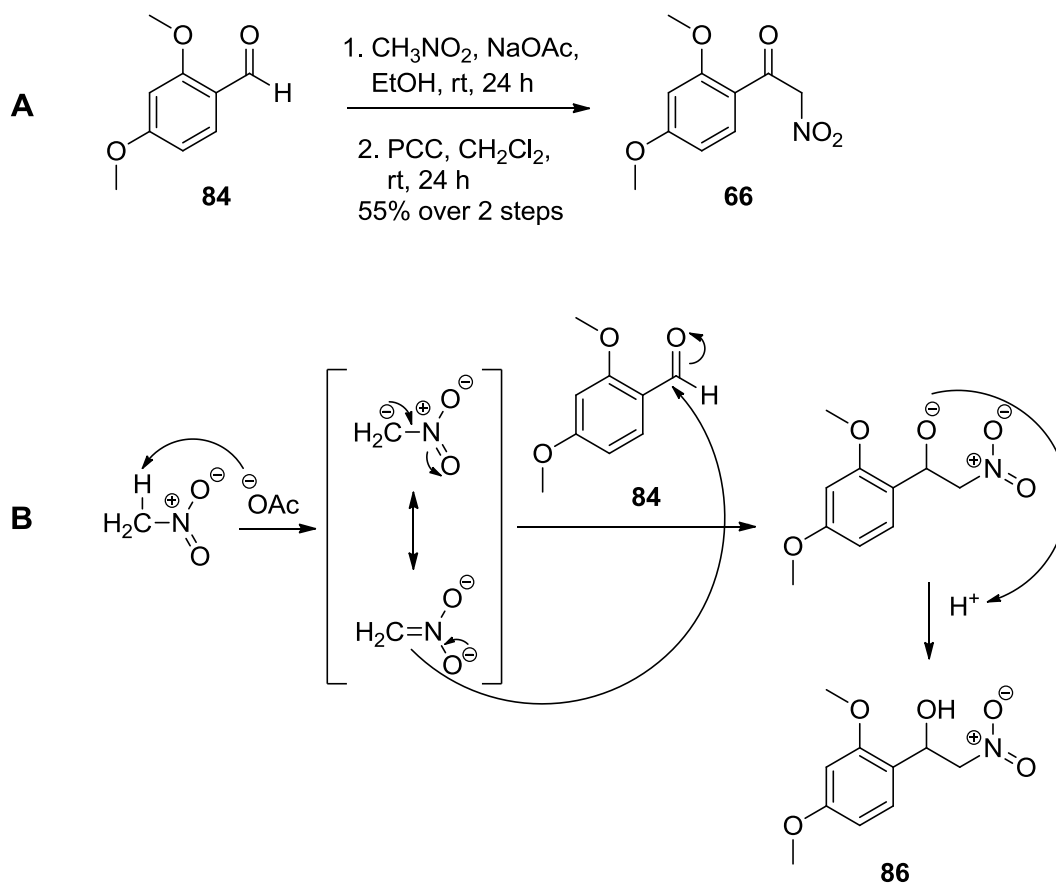
Scheme 26: Synthesis of α -Me-acetophenone **65**.

In order to get α -NO₂-acetophenone **66**, my Bachelor student Franziska Naporra⁷² tried to apply Ballini's one-pot solventless reaction⁷³ shown in Scheme 27. This includes a nitroaldol reaction of CH₃NO₂ and benzaldehyde **84** on activated alumina for 2 h at 0 °C followed by 20 h at rt, then *in situ* addition (0 °C) of wet-alumina supported CrO₃ and standing for additional 20 h at rt before the extraction of the product using Et₂O. Firstly she did the reaction using Al₂O₃-90 active but could not observe any conversion. Upon using Al₂O₃-super 1, she did not get the desired product but 47 % of compound **85** that could be formed by elimination of H₂O from the formed nitroalcohol. Her attempts to oxidize **85** to get the targeted acetophenone **66** using *t*-BuOOH and BuLi⁷⁴ were not successful, which can be attributed to the presence of the electron-donating OMe group in the *ortho* and *para* positions.

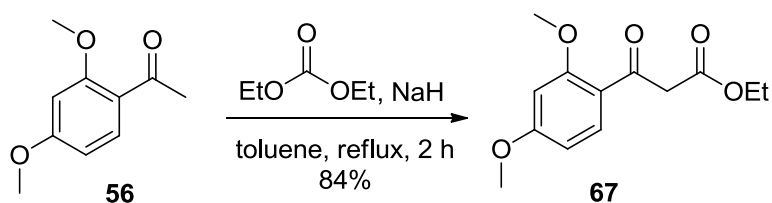


Scheme 27: Attempts to prepare α -NO₂-acetophenone **66** according to Ballini.⁷³

Another two-step procedure was followed then in order to prepare the α -NO₂-acetophenone **66** (Scheme 28A).⁷⁵ The first step was the synthesis of nitroalcohol **86** by a Henry reaction of aldehyde **84** with CH₃NO₂ using NaOAc as illustrated in Scheme 28B. Attempts to purify the nitroalcohol **85** failed which might be rationalized by its instability. Therefore the produced nitroalcohol **86** was subsequently used without further purification in the next step which is oxidation with pyridinium chloroformate (PCC) to give the targeted acetophenone **66** in a 55% yield over the two steps.



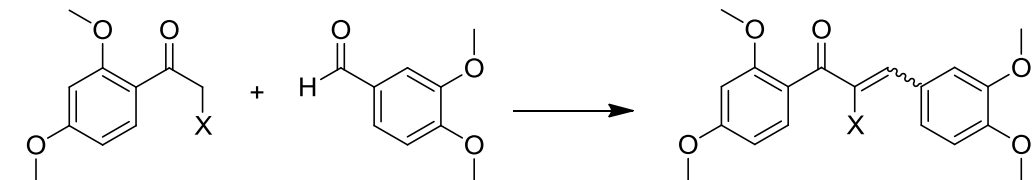
As in the case of α -F- **63** and α -CN-acetophenones **64**, the initial precursor for the 1,3-dicarbonyl compound, α -COOEt-acetophenone **67** was acetophenone **56**. Acetophenone **67** was obtained with a 84% yield by condensation of diethylcarbonate with acetophenone **56** in presence of NaH as shown in Scheme 29.⁷⁶



Synthesis of α -F/CN/Me/NO₂/COOEt-TMCs (68-72):

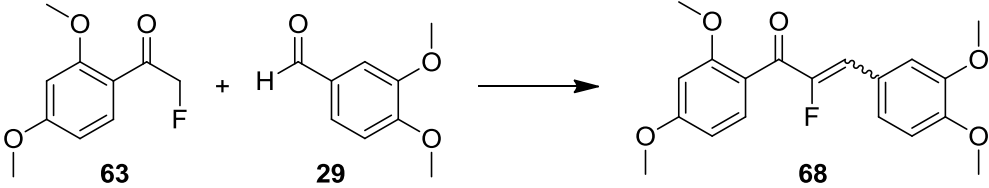
The synthetic conditions for the titled chalcones, which were synthesized from their corresponding acetophenones, are summarized in Table 1 (with the α -H-TMC (**60**)) and the details are given afterwards.

Table 1: Reaction conditions for the synthesis of α -X-TMCs **60**, **68-72** by route 1 in Scheme 23.

 <p>X = H: 56, F: 63, CN: 64, Me: 65, NO₂: 66, COOEt: 67</p> <p>29</p> <p>60, 68-72</p>					
X	Reagents, Solvent	Temp.	Reaction Time	Yield (%)	Product, (configuration)
H	Ba(OH) ₂ · 8 H ₂ O, MeOH	60 °C	24 h	74	α -H-TMC (60), (<i>E</i>)
F	Ba(OH) ₂ · 8 H ₂ O, MeOH	60 °C	24 h	39	α -F-TMC (68), (<i>Z/E</i> 92:8)
CN	piperidine, EtOH	rt	24 h	73	α -CN-TMC (69), (<i>E</i>)
Me	Ba(OH) ₂ · 8 H ₂ O, MeOH	reflux	5 d	41	α -Me-TMC (70), (<i>E</i>)
NO ₂	β -alanine/AcOH, benzene	reflux	24 h	84	α -NO ₂ -TMC (71), (<i>E</i>)
COOEt	piperidine/AcOH, benzene, 4 Å MS	reflux	2 d	71	α -COOEt-TMC (72), (<i>E</i>)

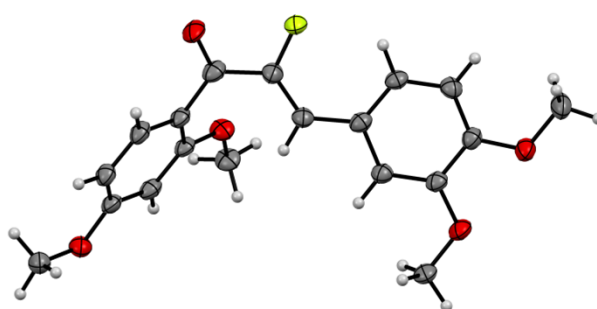
To get the α -F-TMC (**68**), the plan was to use the Claisen-Schmidt condensation of α -F-acetophenone **63** with benzaldehyde **29** in presence of a base. Many bases have been screened by my Bachelor student Frauke Antoni (Table 2).⁷⁷ Firstly piperidine¹³ was tried, but no reaction was observed even at 50 °C. LDA gave also no reaction, while the use of NaH resulted in a low yield. KOH and Ba(OH)₂ · 8 H₂O were the best, therefore α -F-TMC (**68**) was prepared under the same conditions used to synthesize the α -H-chalcones (Scheme 22) to give a 39% yield as a mixture of *Z/E* isomers (92:8).

Table 2: Methods tried to synthesize α -F-TMC (**68**).^{*}

		
Entry	Reaction conditions	Results/Yield of 68 [§]
1	piperidine, EtOH, 50 °C, 3 d	No conversion
2	LDA, THF, rt to 50 °C, 40 h	No conversion
3	NaH, THF, rt, 18 h	25%
4	KOH, EtOH/H ₂ O, 50 °C, 3 d	53%
5	Ba(OH) ₂ · 8 H ₂ O, MeOH, 50 °C, 24 h	62%

^{*} Test reactions were performed by the Bachelor student Frauke Antoni.⁷⁷ The reactions were stopped when the starting material was gone as indicated on TLC, or when no more conversion was observed. [§] calculated using ¹H NMR.

It was possible to get crystals suitable for X-ray analysis upon recrystallization of α -F-TMC (**68**) from EtOAc/petroleum ether using the solvent diffusion method. The X-ray structure (Figure 24) proved its *Z*-configuration and *s-trans* conformation with 54.33° between the two aromatic rings.


Figure 24: X-Ray structure of α -F-TMC (**68**).

The α -CN-TMC (**69**) was prepared from α -CN-acetophenone **64** and benzaldehyde **29** in presence of piperidine¹³ as a base (Table 1). Recrystallization of the product from a mixture of EtOAc/petroleum ether afforded yellow crystals of the pure *E*-isomer of α -CN-TMC (**69**) with a yield of 58%. The X-ray structure is depicted in Figure 25 showing *E*-configuration and the *s-cis* conformation of **69**. A prior structural assignment of an α -CN chalcone as

s-trans conformer based on NOE experiments was published by Lawrence.¹³ But here using the X-ray analysis, the *s-cis* conformation of an α -CN-chalcone was proven for the first time with a dihedral angle of 73.81° between the two aromatic rings. This angle is considerably larger than the published value of 26.88° for the *s-cis* conformer of α -H-TMC (**60**).⁶⁷ The *s-cis* conformation of α -CN-TMC (**69**) could be rationalized by the relative slim CN substituent compared to the halogens (Cl, Br and I, shown later in Figure 27) or a methyl group.

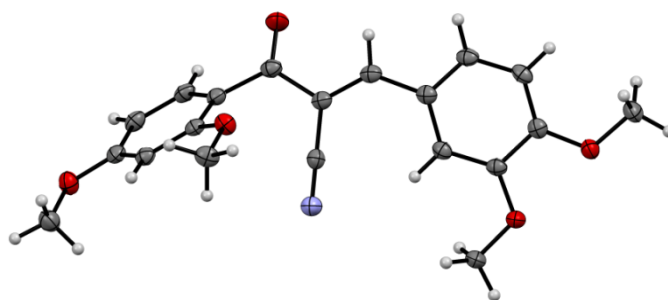


Figure 25: X-Ray structure of α -CN-TMC (**69**).

Like α -F-TMC (**68**), α -Me-TMC (**70**) was also prepared from its corresponding acetophenone **65** and benzaldehyde **29** using $\text{Ba}(\text{OH})_2 \cdot 8 \text{H}_2\text{O}$ in MeOH but at reflux. The reaction gave 41% of the pure targeted chalcone **70**. the *E*-Configuration was assigned to α -Me-TMC (**70**) based on the chemical shift of the β -H in the ^1H NMR spectrum compared with similar chalcones prepared by Ducki.⁷⁸ Ducki's α -Me-chalcones have a chemical shift in the range of 7.08-7.11 ppm for their β -H and were reported to adopt the *E*-configuration and *s-trans* conformation based on an X-ray structure¹² of one of them. Since the shift of the β -H of α -Me-TMC (**70**) is 7.11 ppm, then it was assumed to have the same configuration as that for Ducki's α -Me-chalcones.

To prepare the α -NO₂-chalcone **71**, the nitration method (Scheme 18) of the α -H-TMC (**60**) using nitric oxide⁶⁰ was tried at first but unfortunately no conversion was observed at all. So the other option was to prepare the α -NO₂-acetophenone **66** then to react it with benzaldehyde **29**. This reaction was performed in presence of piperidine/AcOH in benzene⁵⁹ at reflux to afford 84% of the pure *E*-isomer of α -NO₂-TMC (**71**, Figure 26A). The stereochemistry of α -NO₂-TMC (**71**) was assigned as the *E*-isomer based on literature.⁷⁹ Kinugasa *et al.* assigned the *E*-configuration for the α -NO₂-chalcone they prepared according to Dornow's method (Scheme 16) based on IR and UV spectra. They claimed that the synthesis of the α -NO₂-

chalcone is similar to the Knoevenagel condensation of benzaldehyde (**16**) with primary nitroalkanes which is known to give *trans*- β -nitrostyrenes preferentially (Figure 26B).⁷⁹

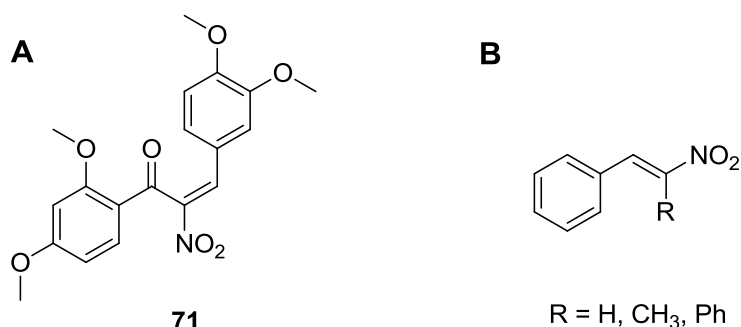
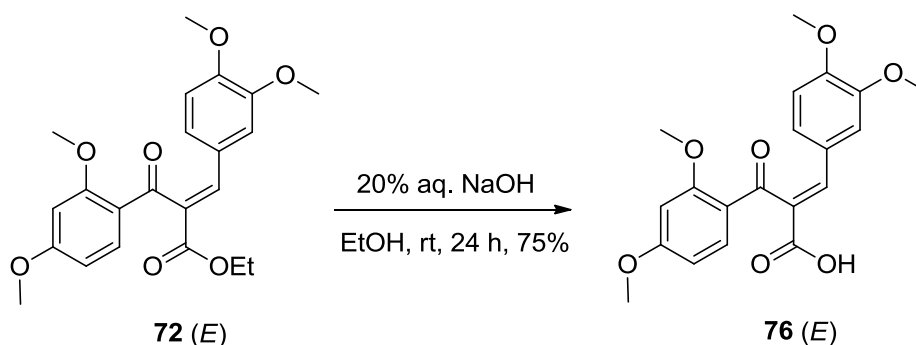


Figure 26: A) (*E*)- α -NO₂-TMC (**71**), B) *trans*- β -Nitrostyrenes formed by Knoevenagel condensation of benzaldehyde (**16**) with primary nitroalkanes.⁷⁹

The α -COOEt-TMC (**72**) was obtained with a 71% yield by Knoevenagel condensation of α -COOEt-acetophenone **67** with benzaldehyde **29** in presence of piperidine/AcOH in benzene that was heated at reflux over molecular sieves (4 Å).⁵³ The stereochemistry of chalcone **72** was assigned as the *E*-isomer based on the ³*J*_{C-H} coupling constants of C_{ketone}-H and C_{ester}-H⁵⁶ as indicated in the introduction (Figure 20).

Synthesis of α -COOH-TMC (**76**)

By saponification of α -COOEt-TMC (**72**) with aqueous NaOH at ambient temperature the acid derivative **76** was obtained in a 75% yield (Scheme 30).⁵⁵ The same double bond configuration (*E*) as for the ester **72** was assumed. It was tried to measure the ³*J*_{C-H} coupling constants of C_{ketone}-H and C_{acid}-H but the peaks of the NMR spectrum were not narrow enough to permit this measurement.

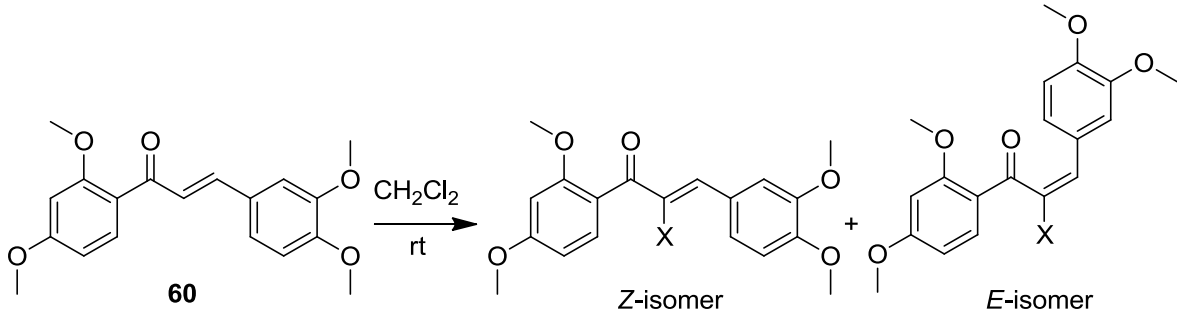


Scheme 30: Synthesis of α -COOH-TMC (**76**).

Synthesis of α -Br/Cl/I-TMCs (**73-75**)

To synthesize the α -halogenated chalcones (**73-75**), it is not possible to follow route 2 (Scheme 23) that was used to prepare the α -F-TMC (**68**). This is because Cl, Br and I substituents are considered as good to very good leaving groups. Hence, **73-75** were prepared following route 2 (Scheme 23) from the α -H-TMC (**60**). At the beginning it was tried to brominate the α -position using the classical bromination method, *i.e.* Br₂/base, but unfortunately this led to bromination on the aromatic rings in addition to the α -position. This could be rationalized by the highly electron-rich aromatic rings bearing the OMe substituents. On the other hand, bromination with Et₄NBr in presence of DMP (**19**, Scheme 8)⁴⁹ gave the desired product **73** in a good yield. This method gave a mixture of *Z* and *E* isomers (as reported in literature)⁴⁹ in about 89% yield, but by recrystallization it was possible to get 58% of the pure *Z*-isomer of compound α -Br-TMC (**73**) (Table 3).

Table 3: Reaction conditions for the synthesis of α -X-TMCs **73-75**.

					
X	Reagents	Time	Yield (%) before rec. ^a	Yield (%) after rec.	Product, (configuration after rec.)
Br ⁴⁹	DMP (1.2 eq.), Et ₄ NBr (1.2 eq.)	24 h	89	58	α -Br-TMC (73), (<i>Z</i>)
Cl	DMP (2.0 eq.), Et ₄ NCl (2.0 eq.)	5 d	29	20	α -Cl-TMC (74), (<i>Z</i>)
I	DMP (2.0 eq.), Et ₄ NI (2.0 eq.)	5 d	nd	29	α -I-TMC (75), (<i>Z/E</i> 93:7)

^a rec: recrystallization

To chlorinate α -H-TMC (**60**) affording the α -Cl analogue **74**, SO₂Cl₂ was first tried according to Edwards.⁴³ The LC-MS analysis of the crude reaction mixture after work-up showed the

presence of the desired product, but the reaction gave a multi-component mixture which made it difficult to get the desired product in a pure form. The idea was then to apply the known procedure used to prepare α -Br TMC (**73**)⁴⁹ to synthesize the α -Cl and α -I compounds **74** and **75** respectively (Table 3) from their corresponding Et₄NX salts. This method gave the desired products (**74** and **75**) although not with high yields.

Recrystallization of chalcones **73** and **74** from EtOAc/petroleum ether gave crystals that were analyzed by X-ray. Solvent-diffusion method was used to get chalcone **75** crystallized. The X-ray structures of chalcones **73-75** (Figure 27) show that they adopt the *Z*-configuration and the *s-trans* conformation. The structural influence of the X group can be found in the dihedral angles between the two aromatic rings of these chalcones. Since the Cl and Br atoms are bigger than the F atom, the dihedral angles between A-ring and B-ring in the case of α -Cl-TMC (**74**) and α -Br-TMC (**73**) were bigger than that of α -F-TMC (**68**) (54.33°, Figure 24). The bulky I-substituent forces the carbonyl functionality and the A-ring more out of the plane leading to a significantly greater dihedral angle of 78.63° for α -I-TMC (**75**).

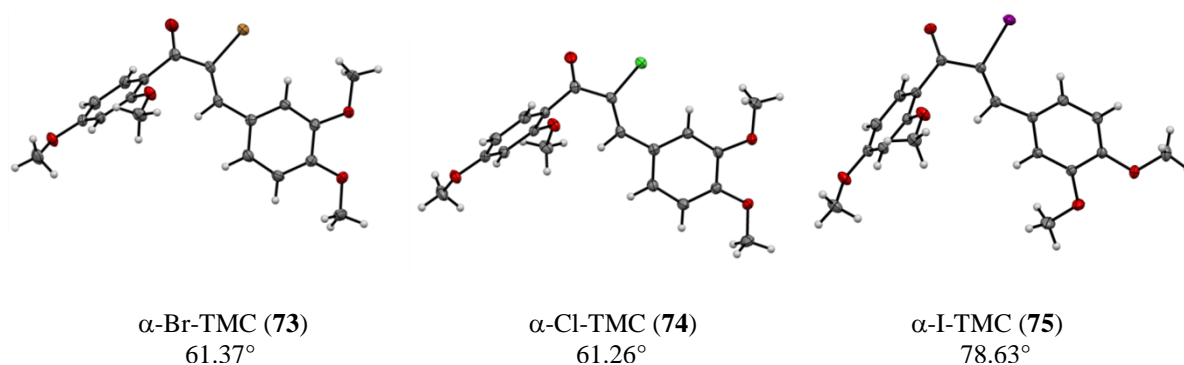
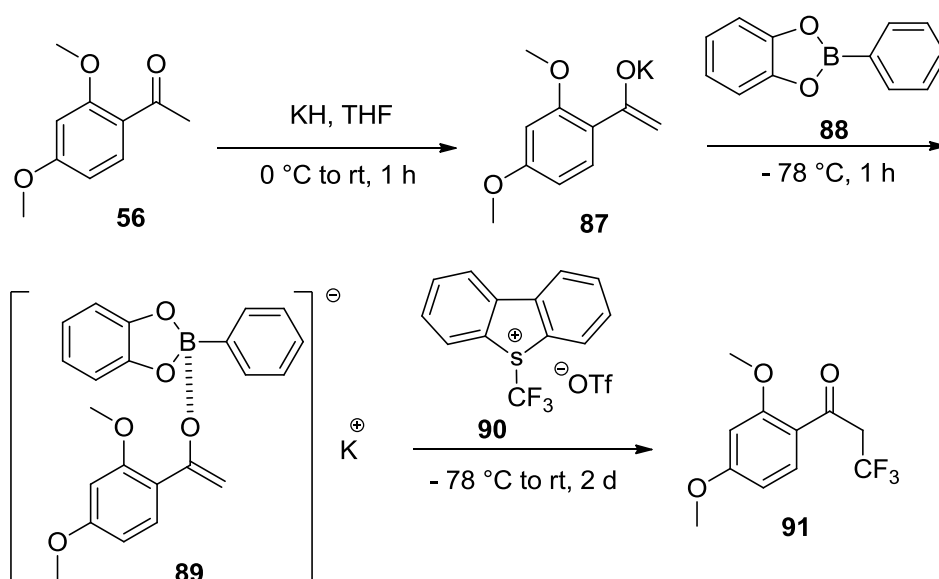


Figure 27: X-Ray structures of α -Br/Cl/I-TMCs (**73-75**). The given angles are the dihedral angles between the two aromatic rings.

Synthesis of α -CF₃-TMC (**77**)

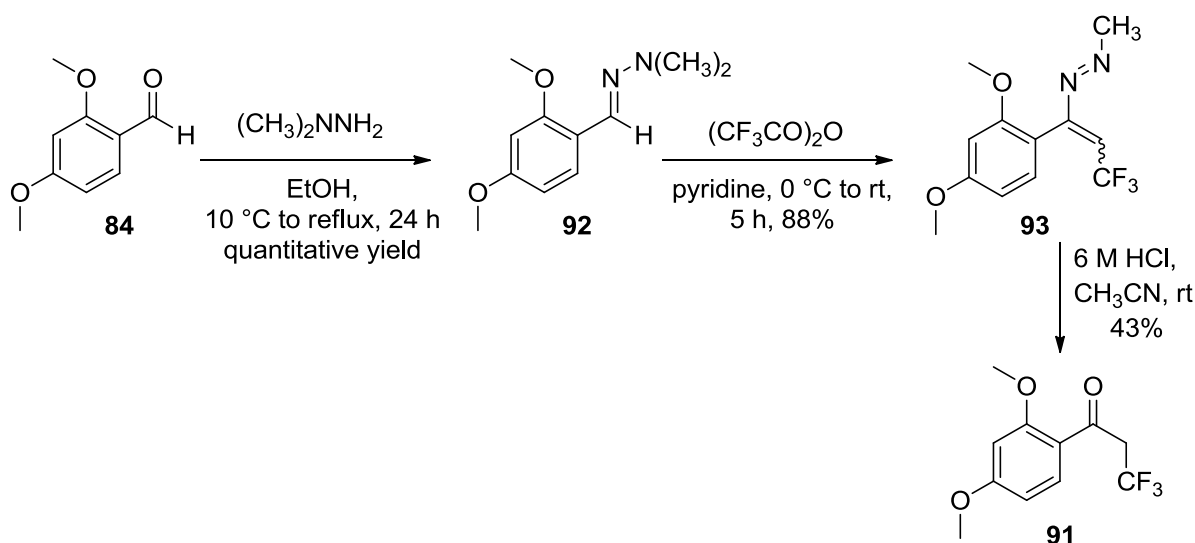
At the time the synthesis of α -CF₃-TMC (**77**) was performed there was no published procedure for a similar chalcone. The first idea to prepare it was by following route 2 (Scheme 23), which implies the synthesis of α -CF₃-acetophenone **91** then its condensation with benzaldehyde **29**. To prepare acetophenone **91**, the method of trifluoromethylation of enolates (Scheme 31)⁸⁰ was tried. Firstly acetophenone **56** was allowed to react with KH to form potassium enolate **87** and then boronic compound **88** was added to act as a Lewis acid that makes a complex **89** with the enolate and thus increasing its nucleophilicity. Finally the commercially available CF₃⁺ source **90** was added to react with the boron complex **89** giving

the acetophenone **91**. Unfortunately no considerable conversion was observed for this reaction sequence.



Scheme 31: The reaction sequence tried to synthesize α - CF_3 -acetophenone **91** by trifluoromethylation of the enolate⁸⁰ form of **56**.

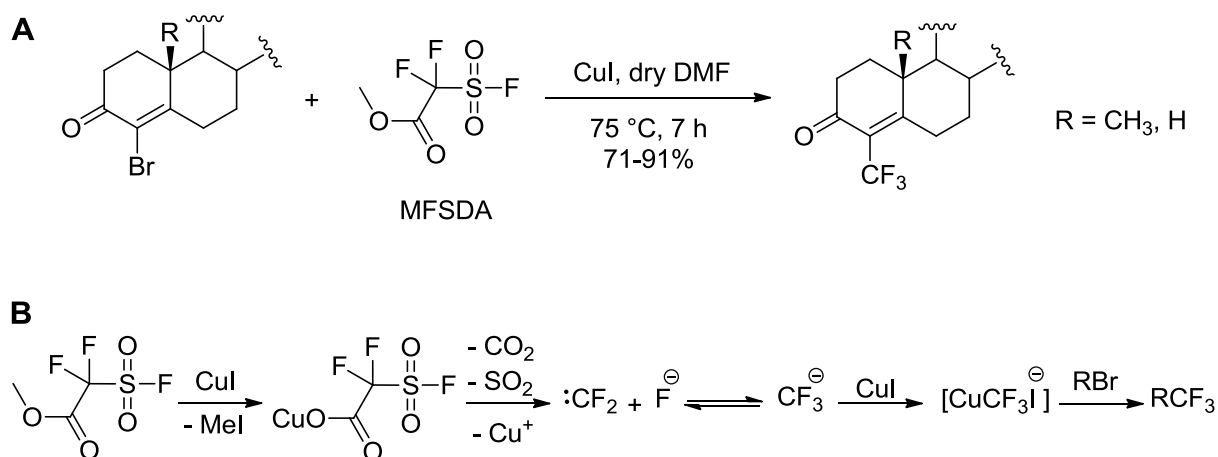
On the other hand, the three-step literature procedure shown in Scheme 32 which was applied to synthesize α - CF_3 -acetophenone **91** gave the desired acetophenone in a good yield (43%). Benzaldehyde **84** was initially converted to hydrazone **92** in a quantitative yield,⁸¹ then hydrazone **92** was treated with trifluoroacetic anhydride ($(\text{CF}_3\text{O})_2\text{O}$) to give compound **93**. The last step was the hydrolysis of **93** to the α - CF_3 -acetophenone **91** under acidic conditions but at rt. No mechanism was given in the reference for the second step.⁸²



Scheme 32: Synthesis of α - CF_3 -acetophenone **91** through the hydrazone **92**.⁸¹⁻⁸²

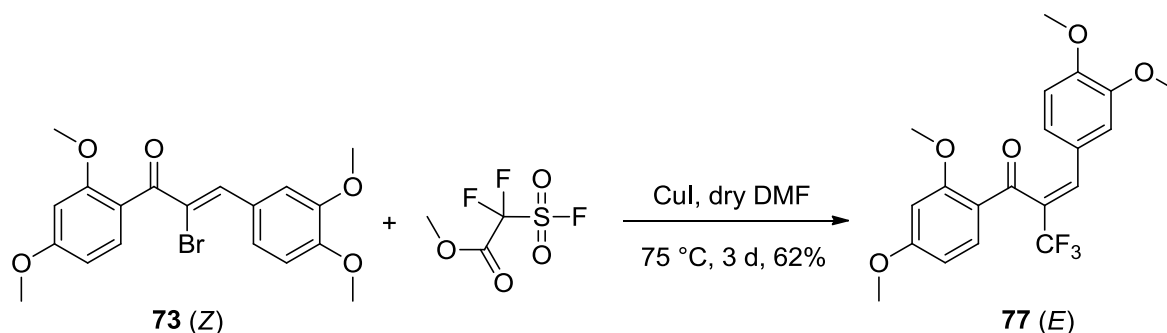
Having α -CF₃-acetophenone **91** in hand, it was tried to perform its condensation with benzaldehyde **29** using Ba(OH)₂ · 8 H₂O as base. The GC-MS measurement showed that the benzaldehyde **29** was not consumed while the CF₃-acetophenone **91** was converted to a compound with a mass of 180 ([M+H]⁺) which cannot be identified. In addition, condensation using piperidine/AcOH in benzene at reflux gave only traces of the desired α -CF₃-chalcone (**77**) that was detectable by only LC-MS analysis. After these unsuccessful trials to get the α -CF₃-chalcone (**77**) following route 2 (Scheme 23), it was thought that route 1 may help to get the desired product.

By searching for a method to trifluoromethylate the α -position of an α,β -unsaturated system, it was found that this was achieved in steroidal molecules utilizing methyl fluorosulfonyldifluoroacetate (MFSDA) in presence of CuI and starting with α -Br steroidal compound as shown in Scheme 33A.⁸³ The proposed mechanism of this trifluoromethylation (Scheme 33B) starts with the formation of Cu-carboxylate with elimination of MeI. The carboxylate salt readily decarboxylates and loses SO₂ affording difluorocarbene (:CF₂) and F⁻ which are in equilibrium with CF₃⁻. The equilibrium is readily shifted to the right in presence of CuI to form the nucleophilic species [CuCF₃I]⁻ that reacts with the α -Br-steroidal alkenyl compound (RBr) affording the α -CF₃ analogue.⁸³



Scheme 33: A) Synthesis of α -CF₃-steroids done by Chen, B) The proposed mechanism for trifluoromethylation of α -Br-steroidal compounds.⁸³

Applying this trifluoromethylation method on α -Br-TMC (**73**) gave α -CF₃-TCM (**77**) in a 62% yield (Scheme 34).



Scheme 34: Synthesis of α -CF₃-TMC (**77**).

The stereochemistry of chalcone **77** was assigned as the *E*-isomer based on its ¹⁹F NMR shift (-62.1 ppm) according to Robins who reported that the ¹⁹F NMR shift* of (*E*)- α -CF₃- α,β -unsaturated compounds (Figure 28) is -65 ± 2 ppm and that of their *Z*-analogues is -58 ± 2 ppm.⁸⁴ In consistent with this fact is the ¹⁹F NMR shift reported for the (*E*)- α -CF₃ chalcone prepared by Cahard (Scheme 19) which is -63.1 ppm.⁶¹

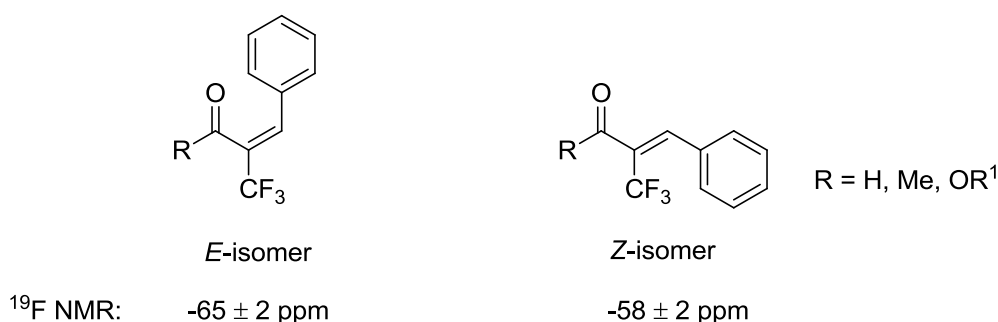
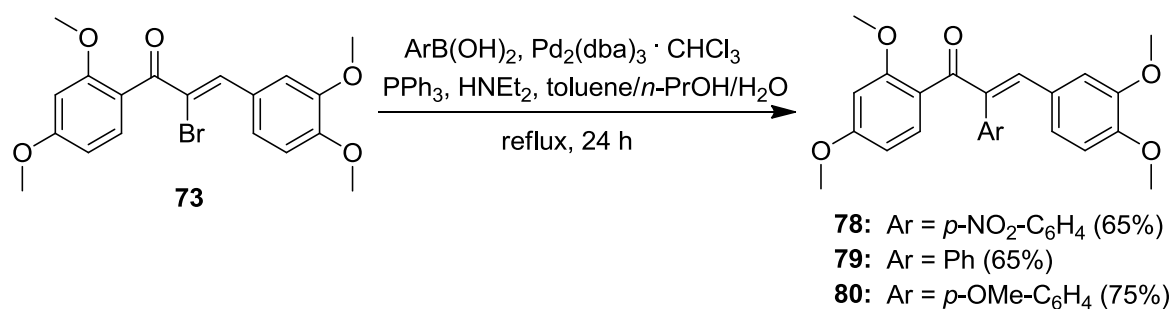


Figure 28: The α -CF₃ compounds prepared by Robinson with their configuration and ¹⁹F NMR shifts.⁸⁴

Synthesis of α -aryl-TMCs **78-80**

Starting with α -Br-TMC (**73**), it was possible to prepare α -aryl-TMCs **78-80** by Suzuki coupling reaction with corresponding boronic acids (Scheme 35).⁴⁸ Three different aryl groups were chosen: Ph, the electron-withdrawing *p*-NO₂-C₆H₄ and the electron-donating *p*-OMe-C₆H₄ group.

* Some literature references reverse the sign convention (*i.e.* negative shifts are reported as positive). Reporting that ¹⁹F NMR chemical shifts are upfield from CCl₃F (the reference) indicates that the values are negative.



Scheme 35: Synthesis of α -aryl-TMCs **78-80**.

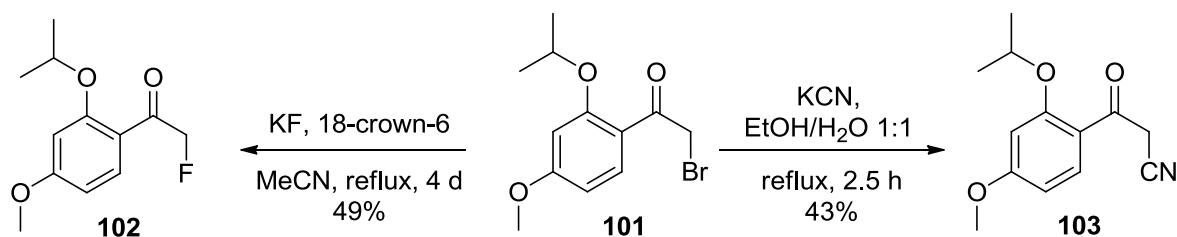
The *E*-pure isomers were separated using preparative TLC plates, but it was found that these compounds have a high tendency to isomerize upon handling and analysis in solution to *E/Z* mixtures. The configuration of the double bond was assigned based on literature data⁴⁸ to be the same as for the starting chalcone **73**, *i.e.* the B-ring and the X group are on the same side of the double bond in both cases.

3.1.3. Synthesis of α -X-2'-hydroxy-3,4,4'-trimethoxychalcones (α -X-HCs)

Due to the enhancement of electrophilicity that can be gained by the presence of an OH group at the 2'-position of chalcones, the aim here is to study the effect of α -substitution in presence of a 2'-OH group. Therefore, a series of α -X-2'-hydroxychalcones (α -X-HCs, X= H (**61**), F (**94**), CN (**95**), Me (**96**), Br (**97**), Cl (**98**), CF₃ (**99**), *p*-NO₂-C₆H₄ (**100**)) was prepared. Two different strategies were followed to get the α -X-HCs. The first one is by using the isopropyl group to protect the 2'-OH in the acetophenones that were used to prepare the 2'-isopropoxy- α -X-chalcones. After that the deprotection of the OH group afforded the targeted α -X-HCs. The second route is the deprotection of the 2'-OH of the TMCs selectively. Here are the details.

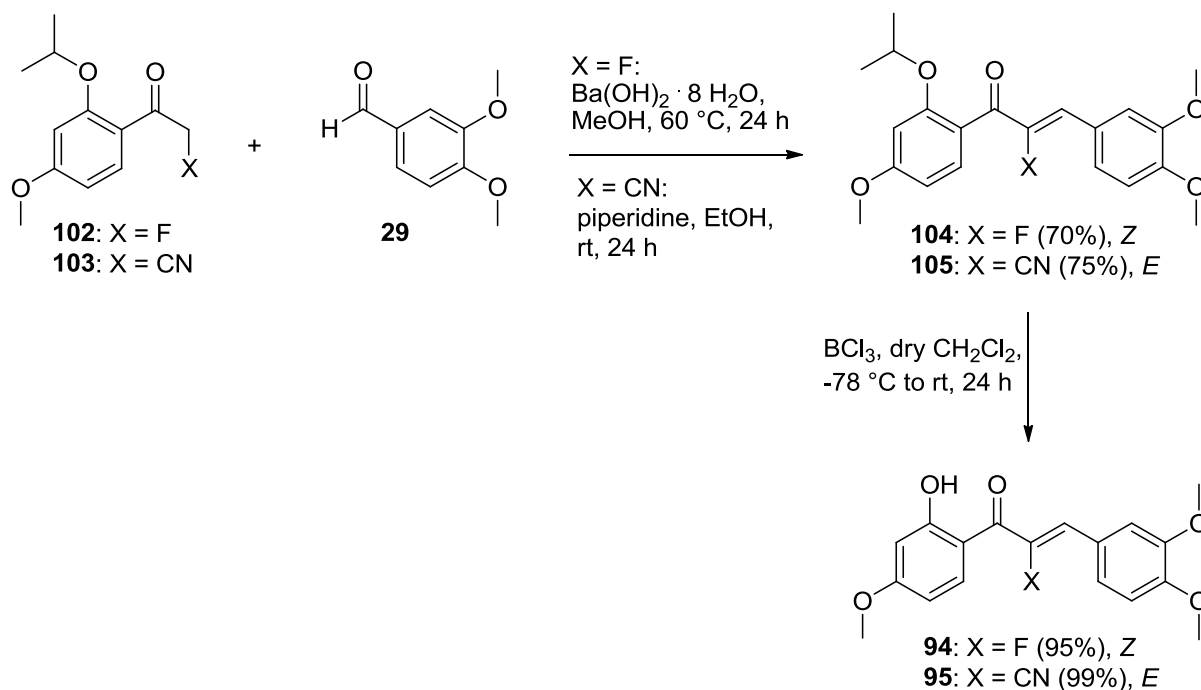
Synthesis of α -F/CN-HCs (**94** and **95**)

In order to prepare α -F-HC (**94**) and α -CN-HC (**95**), the corresponding 2'-protected acetophenones (F **102** and CN **103**) were first synthesized from the Br-acetophenone **101**. These acetophenones were prepared applying the same methods used to synthesize the 2,4-dimethoxy analogues (**63** and **64**) as depicted in Scheme 36 below.



Scheme 36: Synthesis of α -F- and α -CN-acetophenones **102** and **103** respectively.

Subsequently, the Claisen-Schmidt condensation was carried out with benzaldehyde **29** affording the 2'-protected chalcones (α -X-pHC, X = F (**104**) and CN (**105**)) (Scheme 37). Again, Ba(OH)₂ · 8 H₂O was used as a base when X = F and piperidine was used in the case of X = CN to give 70% of α -F-pHC (**104**) and 75% of α -CN-pHC (**105**). The deprotection of the OH functionality using the standard aromatic isopropyl ether-deprotection reagent BCl₃ in CH₂Cl₂ afforded α -F-HC (**94**) in a 95% yield and α -CN-HC (**95**) in almost a quantitative yield.



Scheme 37: Synthesis of α -F- and α -CN-HCs (**94** and **95** respectively).

Figure 29 shows the X-ray structure of α -F-pHC (**104**) with a dihedral angle of 64.46° between the two aromatic rings. This angle is larger than that of the F-TMC analogue **68** (54.33°) which indicates the steric effect of the isopropoxy group in deviating the two aromatic rings more from planarity, hence leading to less conjugation within the π system.

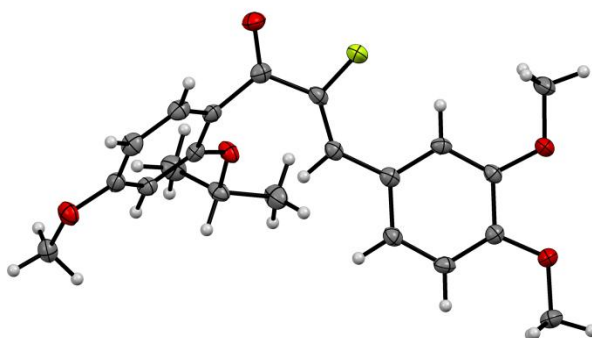
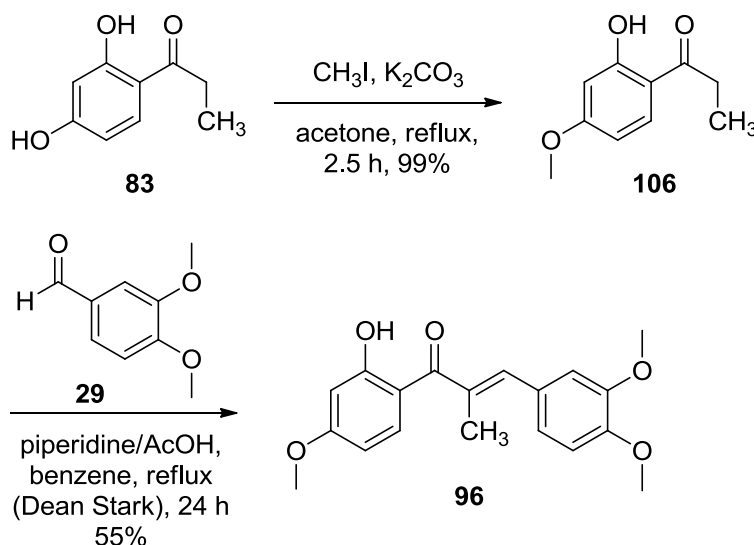


Figure 29: X-Ray structure of α -F-pHC (**104**).

Synthesis of α -Me-HC (**96**)

The synthesis of α -Me derivative **96** was accomplished by Claisen-Schmidt condensation of its corresponding acetophenone **106** with benzaldehyde **29**, no protection was needed here for the 2'-OH group. Selective methylation of the *p*-OH of the commercially available 2,4-dihydroxyacetophenone **83** was firstly performed to get α -Me acetophenone **106** in a quantitative yield.⁷¹ After that, acetophenone **106** was allowed to react with benzaldehyde **29** in presence of piperidine/AcOH in benzene at reflux⁵³ furnishing α -Me-HC (**96**) (Scheme 38).



Scheme 38: Synthesis of α -Me-HC (**96**).

The X-ray structure of chalcone **96** is given in Figure 30. As expected, chalcone **15** adopt the *E*-configuration and the *s-trans* conformation. The dihedral angle between the two aromatic rings is 63.61°. What is noteworthy is the 6-membered ring formed upon H-bonding between the 2'-OH and the carbonyl group.

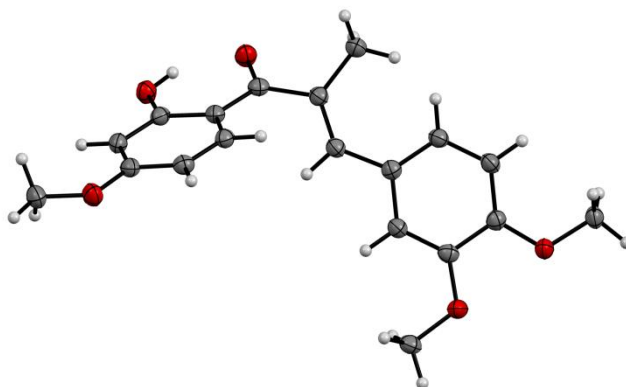
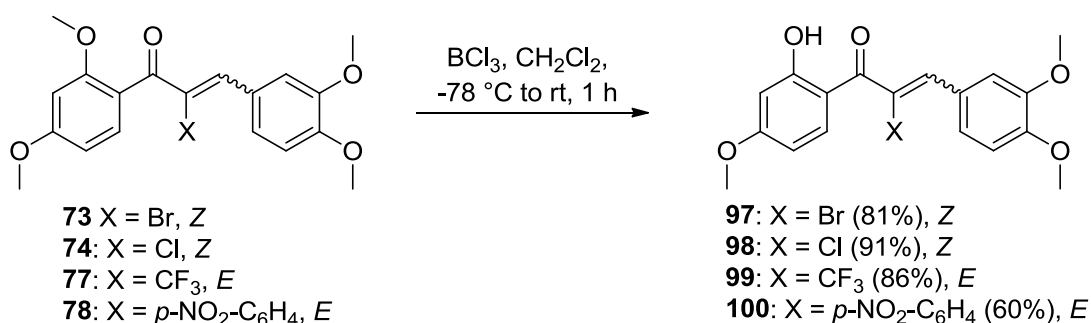


Figure 30: X-Ray structure of α -Me-HC (**96**).

Synthesis of α -Br/Cl/CF₃/*p*-NO₂-C₆H₄-HCs (**97-100**)

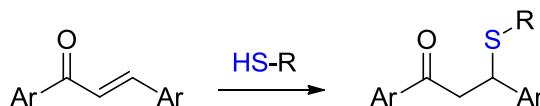
The synthesis of the titled α -X-HCs was accomplished via the selective deprotection of the oxygen at the 2'-position of their α -X-TMCs analogues (Scheme 39). This strategy is based on the fact that the 2'-hydroxy methyl ether is more labile to the BCl₃ than the other methyl ethers which are normally stable in presence of BCl₃. This was found by Veronika Forster while she was trying to deprotect another substituted methoxy-isopropoxychalcone in our group.⁸⁵ Applying this method on α -X-TMCs (X = Br (**73**), Cl (**74**), CF₃ (**77**), *p*-NO₂-C₆H₄ (**78**)) afforded the corresponding α -X-HCs (X= Br (**97**), Cl (**98**), CF₃ (**99**), *p*-NO₂-C₆H₄ (**100**) respectively). It was assumed that the α -X-HCs have the same configuration as their TMCs precursors.



Scheme 39: Synthesis of α -X-HCs from their corresponding α -X-TMCs.

3.2. Assessment of the Michael acceptor activity of chalcones by a kinetic thiol assay

Chalcones are α,β -unsaturated carbonyl compounds which are considered to be good Michael acceptors. This means that their biological activity is based on their ability to react with cysteine residues of the signal-transducing proteins beside their antioxidant properties.² Therefore, assessment of their reactivity in thia-Michael addition reactions (Scheme 40) will be very helpful in predicting and understanding their biological activity. A facile spectrophotometric screening assay to determine the second-order rate constants (k_2) of chalcones in thia-Michael additions was developed by Nafisah Al-Rifai and Sabine Amslinger. This was desirable since there is no reported easy and efficient quantitative method to assess the electrophilicity of different chalcones and other α,β -unsaturated carbonyl compounds.



Scheme 41: Thia-Michael addition to chalcones.

3.2.1. Development of the kinetic thiol assay

The developed assay is based on the use of 96-well microtiter plate which allows for a quick and easy handling together with small amounts of material needed to determine the k_2 values. Since it is expected to have great differences in the reactivity of the tested chalcones, the developed kinetic assay should be able to measure a wide range of reaction rates. In order to develop such an assay, many parameters have to be optimized, such as the solvent system, the pH and the mixing procedure. Screening of solvents (Table 4) showed that some are not good for the solubility and/or for the stability of the tested chalcones and some are volatile so they evaporate during the measurement time. MeCN for example was used by Dinkova-Kostova and Talalay in a 1:1 mixture with 100 mM TRIS-HCl buffer of pH 7.4 (TRIS = tris(hydroxymethyl)aminomethane) to determine the reactivity of several α,β -unsaturated compounds with thiols.²⁴ But when it was tried here, the tested chalcones did not show stability in such a solvent, in addition to its great volatility that makes it inappropriate for long-time measurements. To solve the evaporation problem it was thought that a high boiling solvent is required. DMSO was the first option since it was used by Mayr²⁶ in his kinetic assay to determine the reactivity of some enones towards sulfur ylids and by Appendino²³ in

his NMR experiments of Michael acceptors with thiols. However, when it was tried, the reaction of the tested chalcones with thiols was very slow under these conditions. Other high boiling solvents like diglyme and triglyme were also inappropriate due to their reaction with the plastic of the microplate at high solvent content. The best choice was ethylene glycol which has a high boiling point and where all of the tested compounds have good solubility and stability.

Another way to reduce the evaporation, which is problematic especially for long time measurements, is to cover the 96-well plates with an optical clear PCR foil. The screened solvents were used in combination with 100 mM TRIS-HCl buffer²⁴ of a physiological to slightly basic pH. Different pH's were tried (7.4, 7.6, 7.8, 8.0) and it was found that increasing the pH leads to higher reaction rate, but 7.4 (physiological conditions) was suitable.

Consequently, a solvent system composed of 100 mM TRIS-HCl, pH 7.4, 2 mM EDTA buffer/ethylene glycol in a ratio of 20:80 was the best for the assay. EDTA (ethylenediamine-tetraacetic acid) was used in order to complex with heavy metals that may be present in the solvent and lead to thiol oxidation.⁸⁶

Table 4: Solvents screened for the kinetic thiol assay.

Solvent	Boiling point (°C)	Observations
EtOH	78	Evaporation ↑, solubility ↓
MeOH	65	Stability (chalcone) ↓, evaporation ↑, solubility ↓
MeCN	82	Stability (chalcone) ↓, evaporation ↑, solubility ↓
DMSO	189	Very slow reaction with thiol
Diglyme	162	Microplate was damaged at high solvent content
Triglyme	216	Microplate was damaged at high solvent content
NMP	203	Microplate was damaged at high solvent content
Ethylene glycol	197	Stability (chalcone) ↑, evaporation ↓, solubility ↑

Screened ratios: buffer/organic solvent from 95:5 to 5:95 with 5% steps.

Initially, many thiols (Figure 31) were screened in different buffer and solvent mixtures including cysteamine (**107**), cysteine (**108**), 1,4-butanedithiol (**109**), dithiothreitol (DTT, **110**), 2-mercaptoethanol (**111**) and glutathione (GSH, **112**).

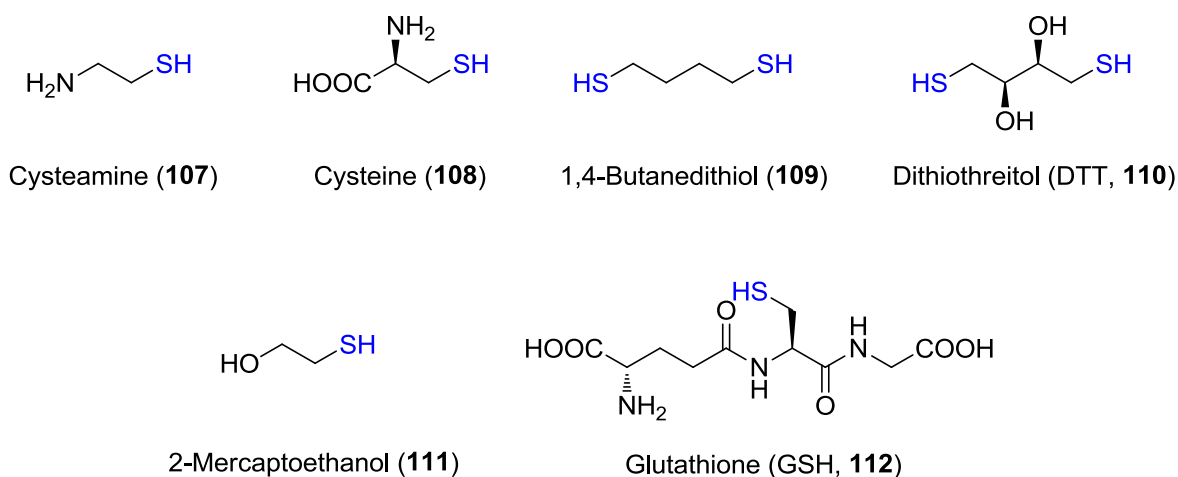


Figure 31: Thiols screened in the kinetic thiol assay.

They displayed diverse reactivities upon reaction with the same chalcone. α -CN-TMC (**69**) for example showed a fast reaction with cysteamine (**107**) and a slow one with 2-mercaptoethanol (**111**) and glutathione (**112**), while the rate of the reaction with 1,4-Butanedithiol (**109**) and dithiothreitol (DTT, **110**) was intermediate (Figure 32).

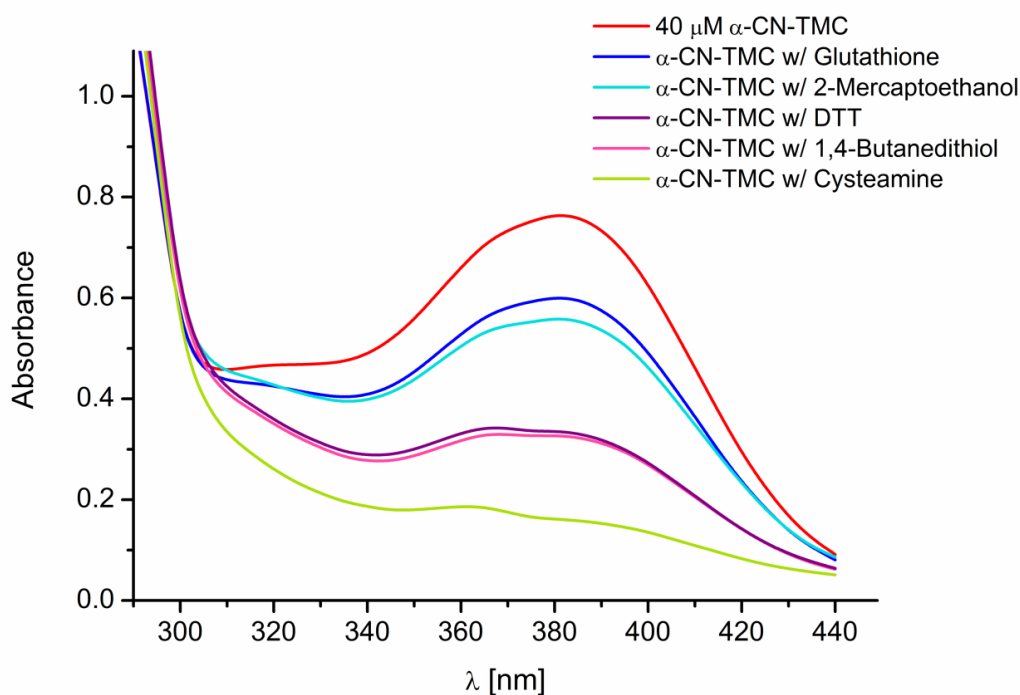
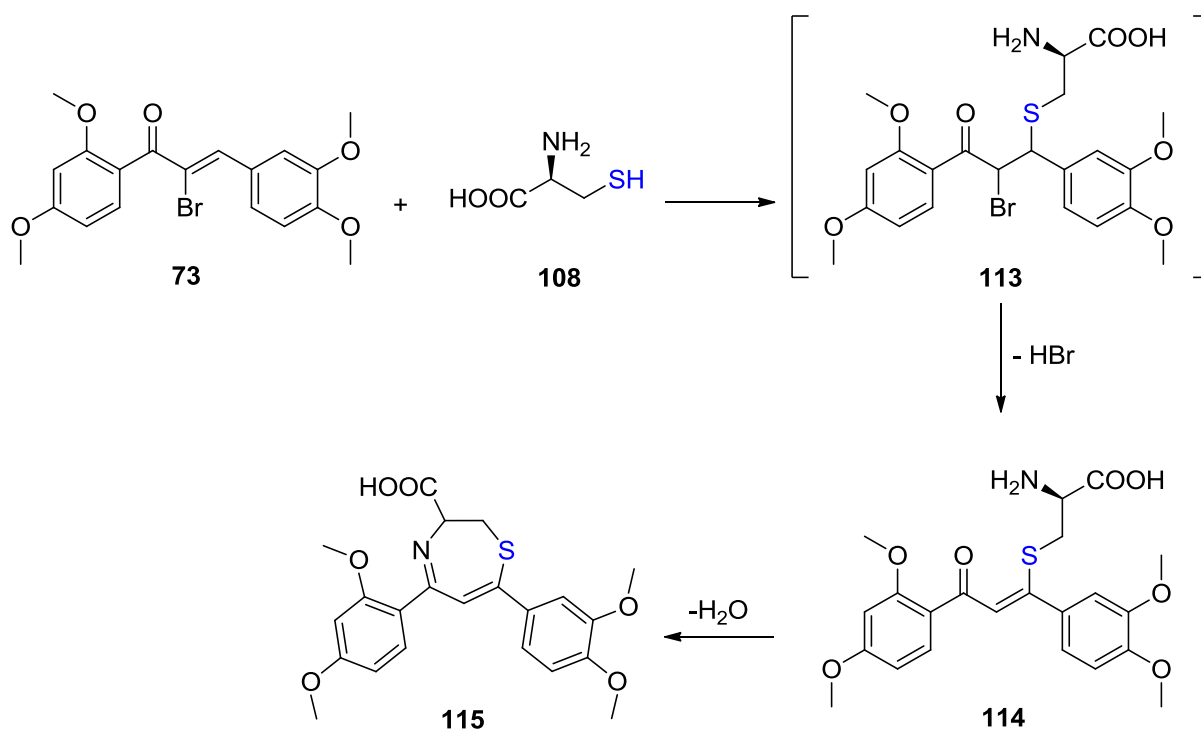


Figure 32: UV-Vis spectra of α -CN-TMC (**69**) without and with different thiols (60 fold) in 100 mM TRIS-HCl pH 7.4, 2 mM EDTA/ethylene glycol (20:80) at 25 °C (in plastic microtiter plate with a PCR foil). The measurement was performed directly after mixing. The measurement was done by Sabine Amslinger.

Cysteine (**108**) was ruled out as a yellow color arose upon its reaction with some of the tested chalcones, such as α -Br-TMC (**73**) that gave the Michael adduct without HBr (**114**) that was formed after elimination of HBr from the Michael adduct **113** and a seven-membered intramolecular condensation product (**115**) (Scheme 41) as was proven by LC-MS analysis. This color made it difficult to find the appropriate wavelength for the kinetic measurement for the chalcones which are also yellow. The high reactivity of cysteamine (**107**) makes it an ideal model thiol for reactivity screening specially that its reactivity is in the same range of many surface thiols of proteins as indicated by its pK_a value (8.3). Cysteamine (**107**) has also proven to be a good nucleophile in thia-Michael additions,²³ while the less reactivity of the other thiols (**109-112**) indicates that they have lower nucleophilicity which might partially⁸⁷ refer to their high pK_a values (1,4-butanedithiol: 10.0; DTT: 9.2; 2-mercaptoethanol: 9.6; glutathione 9.3) and thus lower thiolate concentrations. For all of these reasons, cysteamine (**107**) was picked to assess the Michael acceptor activity of the tested chalcones as will be shown later.



Scheme 42: Reaction of α -Br-TMC (**73**) with cysteine (**108**) (12 fold) in 100 mM TRIS-HCl pH 7.4, 2 mM EDTA/ethylene glycol (20:80) at 25 °C, products were proven by LC-MS measurements.

To test a certain chalcone by the developed kinetic assay, firstly UV-Vis scans with 60 fold of cysteamine (**107**) were performed (Figure 33 as an example) in order to estimate the reactivity range of this chalcone. These scans were used to pick the suitable wavelengths and the appropriate thiol concentrations for the subsequent kinetic measurements. Depending on the

reactivity and the structure of the tested chalcone five thiol concentrations ranging from 12 to 500 fold of the chalcone concentration were chosen for each chalcone/thiol pair. Here are some examples: α -CN-TMC (**69**) was very reactive so the chosen thiol concentrations were 12, 24, 36, 48, 60 fold, α -I-TMC (**75**) showed less reactivity with cysteamine so 60, 72, 84, 96, 108 fold thiol were chosen. It was found that when free phenolic hydroxyl groups were present in the tested chalcone, the picked thiol concentrations should be high enough to avoid the interference of the deprotonation of these groups. Calythropsin **116** for example is a reactive chalcone, but 100-300 fold cysteamine concentrations were used to perform the kinetic measurements in order to gain a first-order exponential decay. The use of lower thiol amounts resulted in a second-order exponential decay which can be rationalized by the deprotonation of the hydroxyl groups of **116** interfering with its reaction with thiol **107**.

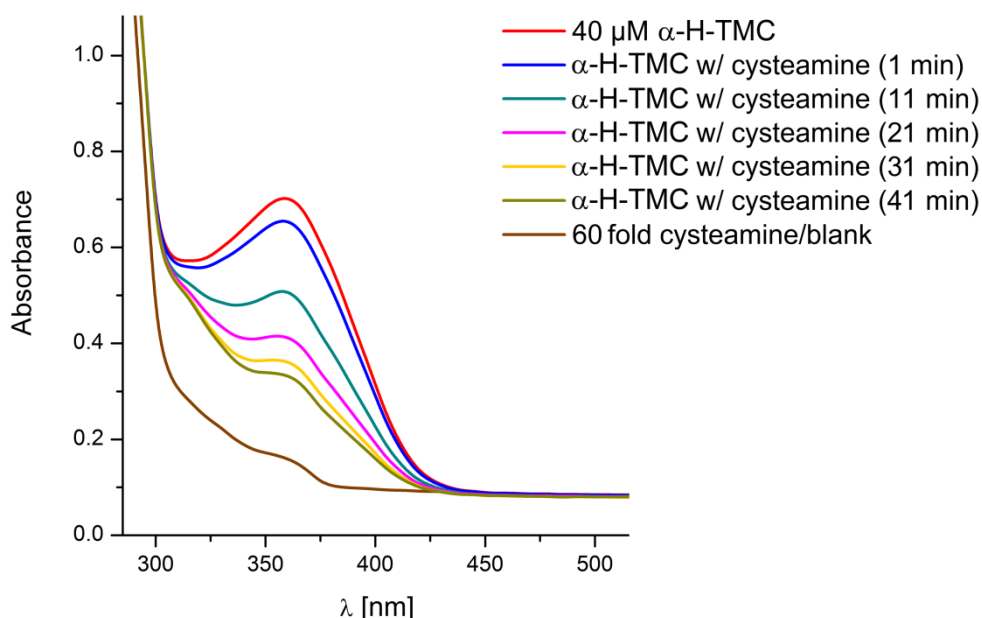


Figure 33: UV-Vis spectra of α -H-TMC (**60**) without and with 60 fold cysteamine (**107**) in 100 mM TRIS-HCl pH 7.4, 2 mM EDTA/ethylene glycol (20:80) at 25 °C (in plastic microtiter plate with a PCR foil).

To be sure that the picked wavelength for each compound was the best, LC-MS measurements of the reaction mixtures were performed. At the appropriate wavelength of each compound, only the decay of the UV-band of the tested compound should occur without an interference with product formation. LC-MS studies were also used to prove the Michael adduct formation, since the isolation of the products was not easy, which could be due to a possible reversibility of the reaction during work-up and purification. Figure 34 shows the LC-MS study for the reaction of α -H-TMC (**60**) with cysteamine (**107**). The LC-MS here proved the existence of chalcone **60** with the Michael adduct **117** that has a mass of 405.1610.

The UV scans of chalcone **60** showed that 360 nm is a good wavelength for the kinetic measurement and UV spectra obtained from the LC-MS measurement (Figure 34-right side) showed that the product **117** is not absorbing at all at this wavelength which implies that this wavelength is a proper wavelength for the kinetic assay.

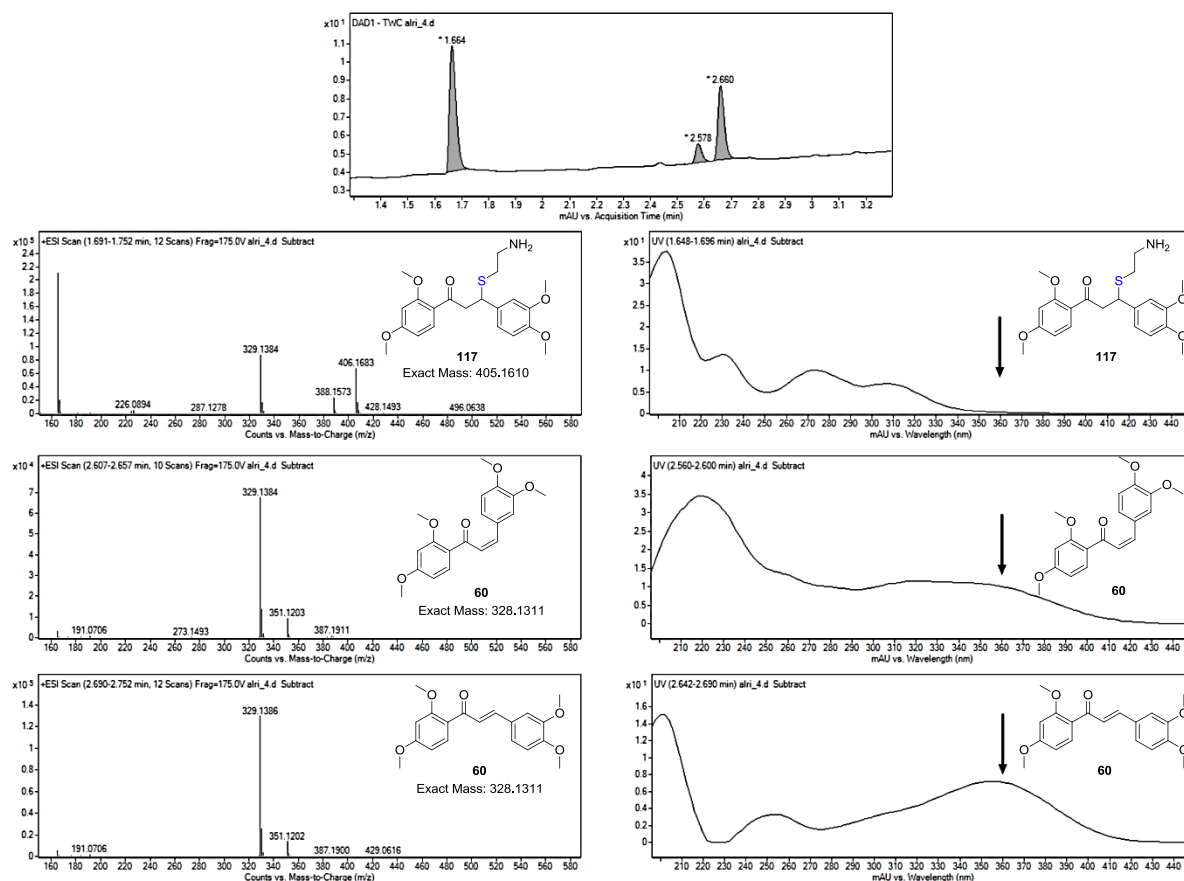


Figure 34: LC-MS analysis of the reaction of α -H-TMC (**60**) with thiol **107** (60 fold) in 100 mM TRIS-HCl buffer pH 7.4, 2 mM EDTA/ethylene glycol (20:80). The top graph shows the HPLC chromatogram with DAD detection (relative quantification). For particular retention times (peaks) the UV scans are displayed with arrows at 360 nm (right side) and the ESI (+ mode) MS spectrum (left side).

Some NMR experiments were also performed to prove the Michael adduct formation independently. ^1H NMR was measured for a solution of α -H-TMC (**60**) in DMSO- d_6 without and with 12 fold of cysteamine (**107**) (Figure 35). The H_α and H_β in the ^1H NMR spectrum of **60** without cysteamine (**107**) (Figure 35A) are located in the region of aromatic hydrogens and show an AB coupling which is typical for the enone hydrogens of the chalcones. In presence of thiol **107**, the AB coupling disappeared and another two new peaks appeared in the aliphatic region (Figure 35B) which means that the addition of cysteamine **107** to the enone system occurred and gave the Michael adduct **117**.

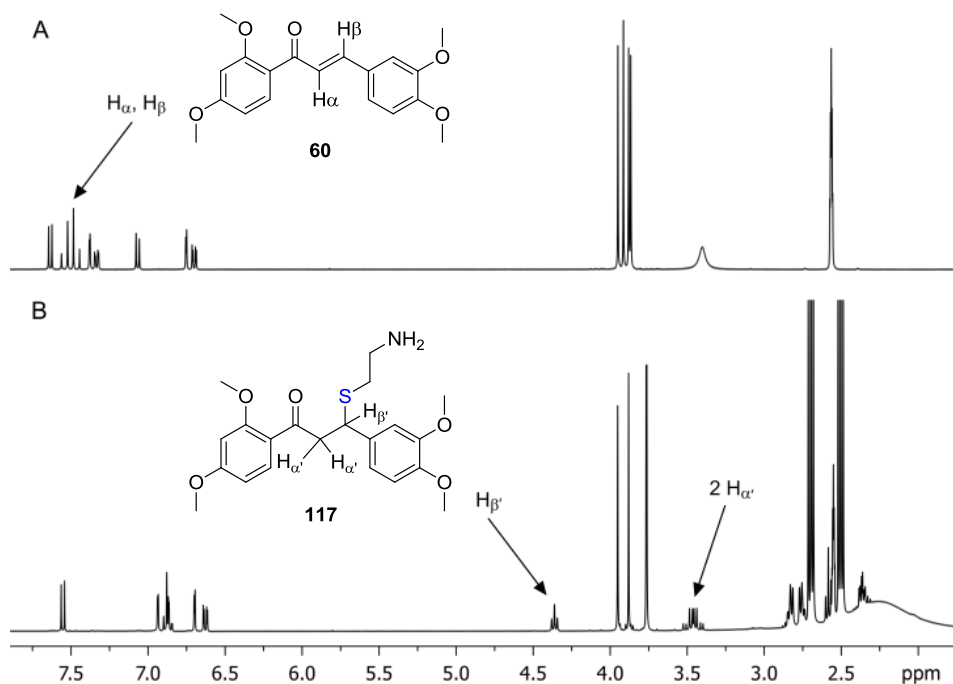


Figure 35: ^1H NMR spectra of α -H-TMC (**60**) in DMSO-d_6 . **A**) without thiol, **B**) with 12 fold cysteamine (**107**) after 5 min.

The results of the aforementioned experiments that were done by Nafisah Al-Rifai and Sabine Amslinger to optimize the conditions of a kinetic thiol assay and to prove the thiol adduct formation are summarized in Figure 36.

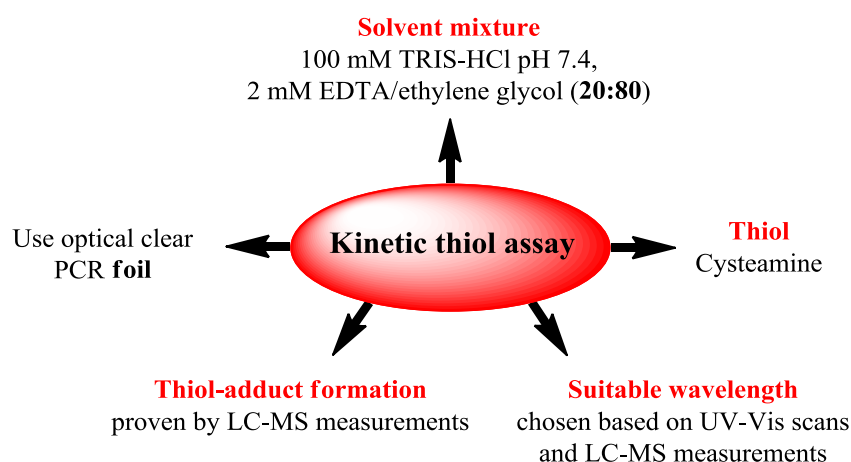


Figure 36: The results of the optimization of the kinetic thiol assay.

3.2.2. The protocol of the developed kinetic thiol assay

Since the kinetic assay was developed, the electrophilicity of chalcones were assessed upon their reaction with thiol **107**. The protocol of this assay starts with the preparation of the

chalcone solution (80 or 160 μM) and the suitable thiol dilutions in the buffer/ethylene glycol (20:80). Then they are pipetted in the 96-well microplate in pre-measurement wells: one row for the chalcone solution and another one for the thiol dilutions. Afterwards, the plate is incubated for 10 min at 25 °C inside the spectrophotometer. Using a multichannel micropipette, equivalent volumes of chalcone solutions and thiol solutions are combined and mixed thoroughly in a deep-well microplate covered with a foil. This way of mixing was chosen since the shaking inside the spectrophotometer was not efficient. After mixing, the reaction mixture is pipetted in the measuring 96-well plate which is then covered with a PCR foil. Lastly the measurement is started to get the decay of absorbance of the chalcone versus time. Measurements are done in duplicates and for each compound 3-7 independent experiments are performed.

For very fast reactions stopped-flow technique (Figure 37) was conducted since this technique is based on a rapid mixing of the two-reactant solutions. The measurements were performed at 25 °C in the same solvent system used in the microtiter plate assay (100 mM TRIS-HCl buffer pH 7.4 with 2 mM EDTA/ethylene glycol (20:80)).

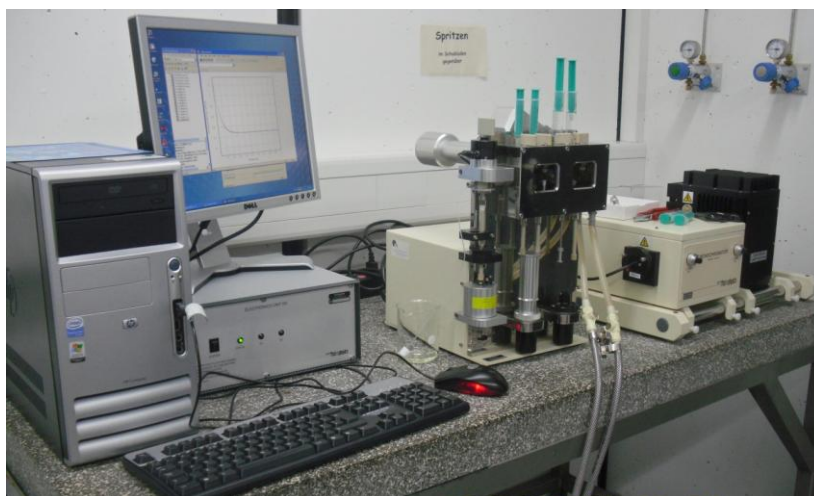
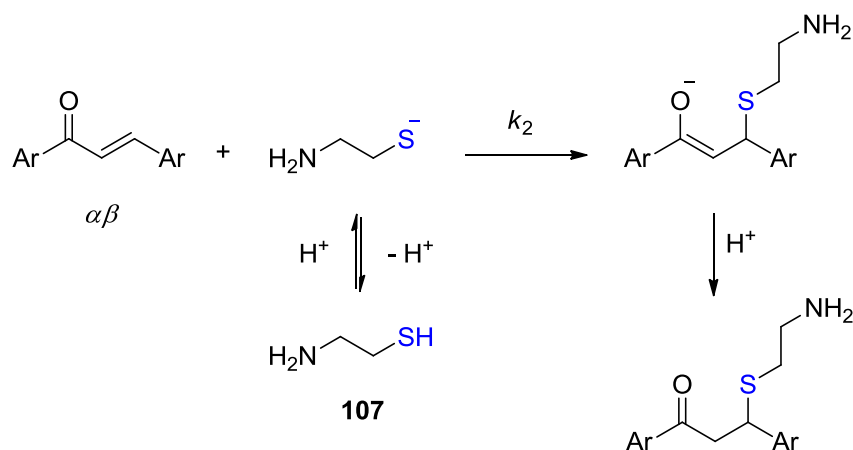


Figure 37: Stopped-flow instrument.

3.2.3. Determination of the second-order rate constant for reaction of a chalcone with a thiol

The idea of the developed assay is to measure the second-order rate constant (k_2) for the reaction of a certain chalcone with a thiol such as cysteamine (**107**) (Scheme 42). To achieve this, the measurements were conducted in presence of an excess of the thiol so that the pseudo-first-order kinetics can be applied as shown below.



Scheme 43: Reaction of chalcones with cysteamine (**107**) relevant to the kinetic assay.

The reaction rate equation for the two reactants ($\alpha\beta$ and *Thiol*) in Scheme 42 is:

$$\text{Rate} = -\frac{d[\alpha\beta]}{dt} = k_2[\alpha\beta][\text{Thiol}] \quad (1)$$

Since the thiol is in excess, its concentration can be absorbed within the rate constant (k_2), obtaining a pseudo-first-order constant (k_{obs}) that depends on the concentration of thiol only (Equations 2-4).

$$[\text{Thiol}] \gg [\alpha\beta] \rightarrow [\text{Thiol}] = \text{const.} = [\text{Thiol}]_0 \quad (2)$$

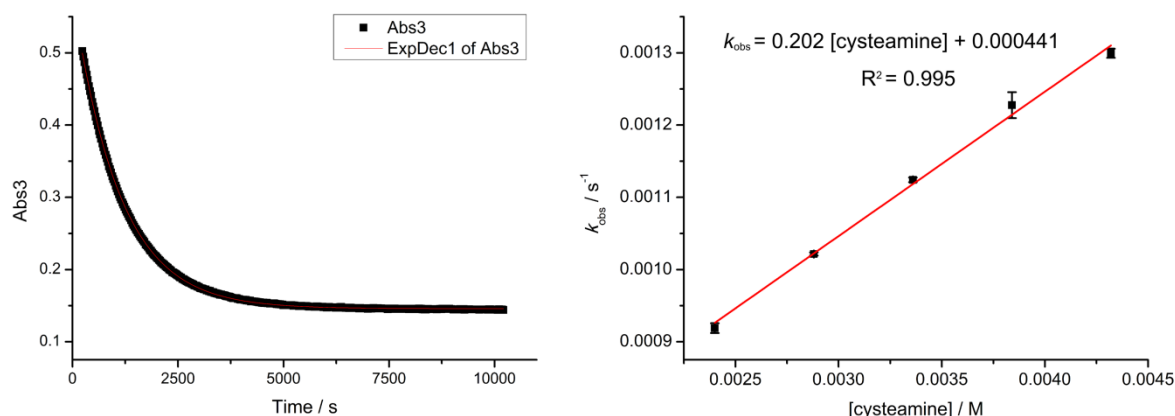
$$\text{So } \text{Rate} = k_{\text{obs}}[\alpha\beta] \quad (3)$$

$$\text{with } k_{\text{obs}} = k_2[\text{Thiol}]_0 \rightarrow k_2 = \frac{k_{\text{obs}}}{[\text{Thiol}]_0} \quad (4)$$

In order to calculate the second-order rate constant k_2 values, firstly the obtained raw data are corrected versus the blank to give individual curves (A_t vs. time) for each measurement. Then the k_{obs} values are gained from the time-dependent decay of the absorbance (A_t) of the $\alpha\beta$ (chalcone) with *Thiol* by fitting the data of individual experiments to the first-order exponential equation (5):

$$A_t = A_0 e^{-k_{\text{obs}}t} + C \quad \text{with } A_0 = A_t([\alpha\beta]_0) \quad (5)$$

with the software OriginPro 8. Finally the k_2 values are obtained by linear regression of the k_{obs} values versus their corresponding thiol concentrations. Figure 38 shows how the k_2 value was determined for the reaction of α -H-TMC (**60**) with cysteamine (**107**) as an example.



Entry	Fold cysteamine	Cysteamine [M]	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean of duplicates
Abs3	60	0.00240	9.12 x 10 ⁻⁴	9.19 x 10 ⁻⁴
Abs4			9.26 x 10 ⁻⁴	
Abs5	72	0.00288	1.02 x 10 ⁻³	1.02 x 10 ⁻³
Abs6			1.02 x 10 ⁻³	
Abs7	84	0.00336	1.12 x 10 ⁻³	1.12 x 10 ⁻³
Abs8			1.13 x 10 ⁻³	
Abs9	96	0.00384	1.25 x 10 ⁻³	1.23 x 10 ⁻³
Abs10			1.21 x 10 ⁻³	
Abs11	108	0.00432	1.31 x 10 ⁻³	1.30 x 10 ⁻³
Abs12			1.29 x 10 ⁻³	
$k_2 = 0.202 \text{ M}^{-1} \text{ s}^{-1}$				

Figure 38: Left graph: Exponential decay curve for the reaction of 40 μM of $\alpha\text{-H-TMC}$ (**60**) with 60 fold cysteamine (**107**) at 360 nm in 100 mM TRIS-HCl pH 7.4, 2 mM EDTA/ethylene glycol (20:80) which represents the first entry in the table below. Right graph: k_2 determination for the reaction of $\alpha\text{-H-TMC}$ (**60**) with cysteamine (60-108 fold). The table shows the k_{obs} values obtained at each thiol concentration as duplicates, then the mean of duplicates and finally the k_2 value.

In the case of stopped-flow measurements, the k_{obs} values are given directly by the software of the instrument. So one has only to do the linear fits to get the k_2 values.

3.2.4. Assessment of the reactivity of $\alpha\text{-H-chalcones}$

First of all, the reactivity of some $\alpha\text{-H-chalcones}$ was assessed by the developed kinetic assay since almost all natural product chalcones are α -unsubstituted – $\alpha\text{-OH-chalcones}$ are the only exception.⁸⁸ In order to establish structure-activity relationships, 2',3,4,4'-tetrasubstituted chalcones were mainly chosen, such as the natural chalcones butein (**2**) and calythrpsin (**116**). Chalcone (**1**), flavokawain A (**3**), isoliquiritigenin (**4**) and xanthohumol (**5**) (Figure 6) were also tested. Table 5 shows the parameters used in the kinetic measurements of the tested $\alpha\text{-H-chalcones}$, *i.e.* wavelengths, fold cysteamine (**107**) and time intervals (Δt) and the results

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of the kinetic studies are given afterwards in Table 6. Representative k_2 value determinations for the tested α -H-chalcones are given in the Appendix.

Table 5: Wavelengths, fold thiol and time intervals (Δt) used in the kinetic thiol assay of α -H-chalcones.

#	Name	Wavelength [nm]	Fold cysteamine ^a	Δt [s]
1	Chalcone	320	12-60	11
62	2'-Hydroxychalcone	295	100-500	Stopped-flow
2	Butein	390	60-108	
3	Flavokawain A	375	60-108	11
4	Isoliquiritigenin	390	60-108	17
5	Xanthohumol	390	60-108	50
60	2',3,4,4'-Tetramethoxy-chalcone	360	60-108	25
61	2'-Hydroxy-3,4,4'-trimethoxy-chalcone	390	60-108	11
116	Calythropsin	390	100-300	11
118	4-Methoxy-2',3,4'-trimethoxy-chalcone	390	60-108	18
119	2',4'-Dihydroxy-3,4-dimethoxy-chalcone	390	60-108	15
120	2',3,4,4'-Tetraisopropoxy-chalcone	365	60-108	35
121	4'-Methoxy-2',3,4-triisopropoxychalcone	365	60-108	32
122	2',4,4'-Triisopropoxychalcone	360	60-108	35
123	4-Methoxy-2',3,4'-triisopropoxychalcone	360	36-84	40
124	2', 4'-Diisopropoxy-3,4-dimethoxychalcone	365	60-108	40

^a **12-60:** 12, 18, 24, 36, 48, 60; **36-84:** 36, 48, 60, 72, 84; **60-108:** 60, 72, 84, 96, 108; **100-300:** 100, 150, 200, 250, 300; **100-500:** 100, 200, 300, 400, 500.

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Table 6: k_2 Values of α -H-chalcones with cysteamine (**107**) at 25 °C.*

#	2'	3'	4'	6'	3	4	k_2^a [$\text{M}^{-1} \text{s}^{-1}$]
1	H	H	H	H	H	H	3.04 ± 0.10
62	OH	H	H	H	H	H	5.08 ± 0.043^b
2	OH	H	OH	H	OH	OH	0.271 ± 0.027
3	OH	H	OMe	OMe	H	OMe	0.649 ± 0.0090
4	OH	H	OH	H	H	OH	0.258 ± 0.010
5	OH	prenyl	OMe	H	H	OH	0.124 ± 0.0054
60	OMe	H	OMe	H	OMe	OMe	0.193 ± 0.019
61	OH	H	OMe	H	OMe	OMe	0.717 ± 0.041
116	OH	H	OMe	H	OH	OH	0.325 ± 0.011
118	OH	H	OH	H	OH	OMe	0.417 ± 0.0079
119	OH	H	OH	H	OMe	OMe	0.464 ± 0.039
120	OiPr	H	OiPr	H	OiPr	OiPr	0.135 ± 0.0047
121	OiPr	H	OMe	H	OiPr	OiPr	0.148 ± 0.0083
122	OiPr	H	OiPr	H	H	OiPr	0.108 ± 0.0056
123	OiPr	H	OiPr	H	OiPr	OMe	0.118 ± 0.0069
124	OiPr	H	OiPr	H	OMe	OMe	0.103 ± 0.0082

^a Reactions were carried out in 100 mM TRIS-HCl pH 7.4, 2 mM EDTA/ethylene glycol (20:80) under pseudo-first order conditions at a concentration of 40 μM for chalcones and 12 to 500 fold cysteamine (**107**).

^b Done by stopped-flow technique. * The measurements and data analysis were done by Nafisah Al-Rifai, Sabine Amslinger and Katrin Winter.

A good structure-activity relationship can be derived from the k_2 values in Table 6. The main points are indicated in Figure 39 and the details are discussed afterwards.

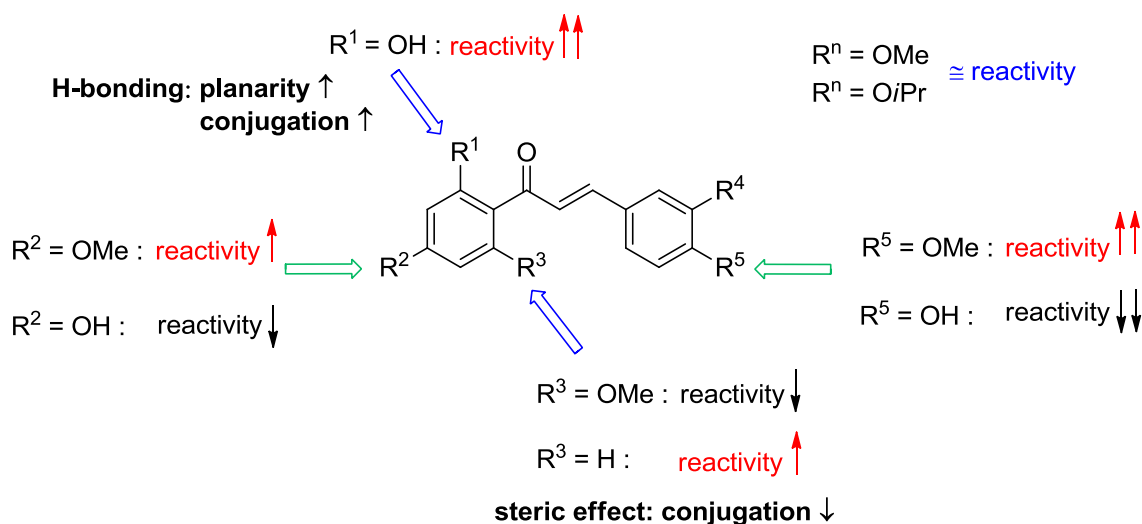


Figure 39: The structure-activity relationship deduced from the k_2 values of α -H-chalcones in Table 6.

1. The 2'-OH enhances the reactivity significantly which is consistent with the literature.⁸⁹⁻⁹⁰ Upon reaction with cysteamine (**107**), 2'-hydroxychalcone (**62**) has a k_2 value of $5.08 \text{ M}^{-1}\text{s}^{-1}$ being the most active compound in the series. It is more reactive than its parent compound chalcone (**1**, k_2 : $3.04 \text{ M}^{-1}\text{s}^{-1}$) by about 1.7 fold. The influence of the 2'-OH can also be seen when the 2'-OH chalcone **61** (k_2 : $0.717 \text{ M}^{-1}\text{s}^{-1}$) is compared with its 2'-OMe analogue **60** (k_2 : $0.193 \text{ M}^{-1}\text{s}^{-1}$). The 2'-OH affects the reactivity of the chalcones by activating their carbonyl group through the intramolecular H-bonding and by stabilizing the conjugation leading to less distortion from planarity. This is indicated by the smaller dihedral angle between the two rings in α -H-HC (**61**, Figure 23) compared to α -H-TMC (**60**)⁶⁷ as mentioned before.
2. The least reactive compound in the 2'-OH chalcone series is xanthohumol (**5**) with a k_2 value of $0.124 \text{ M}^{-1}\text{s}^{-1}$. This is due to its very electron-rich and sterically demanding A-ring.
3. The OH substituent is considered to be more electron-donating than the OMe group due to its negatively charged state upon deprotonation. Therefore replacing the OH substituent with an OMe increased the reactivity. It was noticed that such a replacement in the B-ring enhanced the reactivity more than a similar one in the A-ring, since the substituents on the B-ring are closer to the double bond of the enone moiety and thus have more resonance and inductive effects on the conjugation of the π system. This point was deduced from the comparison of k_2 values of chalcone **2** with chalcones **116** and **118**. Chalcone **116** (k_2 : $0.325 \text{ M}^{-1}\text{s}^{-1}$) with a 4'-OMe showed more reactivity than its

tetrahydroxy analogue **2** (k_2 : $0.271 \text{ M}^{-1}\text{s}^{-1}$) and chalcone **118** (k_2 : $0.417 \text{ M}^{-1}\text{s}^{-1}$) which is a 4-OMe analogue is even much more reactive than chalcone **116**.

4. Butein (**2**) and its 3-dehydroxy analogue isoliquiritigenin (**4**) have unexpectedly nearly the same k_2 values which indicates a particularly big influence of the substituents in the 4-position.
5. There is only a small difference in reactivity between the two regioisomers flavokawain A (**3**) (k_2 : $0.649 \text{ M}^{-1}\text{s}^{-1}$) and α -H-HC (**61**) (k_2 : $0.717 \text{ M}^{-1}\text{s}^{-1}$) which both have three OMe groups. This could be a result of the steric hindrance of the 6'-OMe in **3** that leads to less conjugation and thus less reactivity compared to chalcone **61**.
6. The reactivity of the isopropoxy compounds **120-124** is somewhat similar to their tetramethoxychalcone analogue **60**. This is not surprising since they are all tetraalkoxy compounds. The slight variation in their reactivity might be due to steric factors which affect the dihedral angle between the two aromatic rings thus affecting the conjugation of the π system.

Overall, the reactivity of the chalcones is affected by the electronic nature of the substituents on the aromatic rings. Simultaneously the steric effects of these substituents play an essential role.

The formation of the expected thiol adduct **A** (Figure 40) in the kinetic assay of the compounds in Table 5 was proven in all cases by LC-MS analysis of the assay samples. The chromatograms of LC-MS analyses of the tested α -H-chalcones are shown in the Appendix.* In some cases, a seven membered 1,4-thiazepine intramolecular condensation product **B** was found in addition to product **A**. The cyclized product **B** which is formed by the nucleophilic attack of the NH_2 residue on the carbonyl group was found only when a 2'-OH was present – with the exception of butein (**2**) and flavokawain A (**3**). Such a reaction was reported recently for α,β -unsaturated aldehydes.²³

* The LC-MS results of the chalcones that were tested by Katrin Winter are not included in the Appendix.

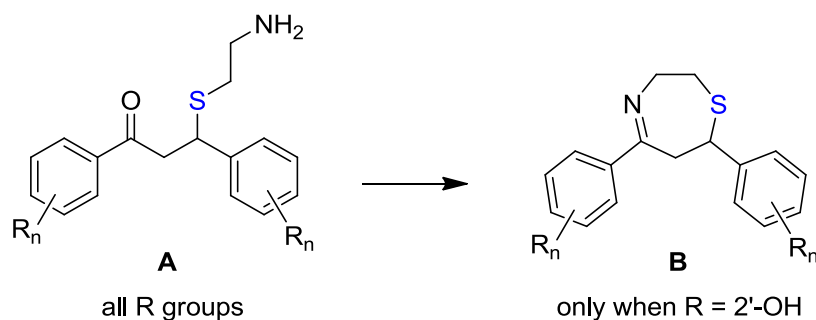


Figure 40: Products of the reaction of α -H-chalcones with cysteamine (**107**) in 100 mM TRIS-HCl pH 7.4, 2 mM EDTA/ethylene glycol (20:80) at 25 °C (found by LC-MS analysis).

It was tried to determine the k_2 value for phenylvinylketone (Figure 41) which is an α,β -unsaturated compound with only one aromatic ring, but the attempts were unsuccessful because its absorption maximum is less than 300 nm and the developed UV-Vis detection-based assay cannot be used in such a case. This can be considered as a limitation of the assay developed.

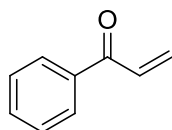


Figure 41: Structure of phenylvinylketone.

3.2.5. Assessment of the reactivity of α -X-TMCs

The chemical reactivity of the synthesized α -X-TMCs towards thiols was determined using the developed spectrophotometric kinetic assay. This allows for a deeper insight into their potential biological activities that is based on the Michael addition of thiols to their enone unit as will be shown afterwards.

The UV-Vis spectra of α -X-TMCs (Figure 42) which were obtained prior to their reactivity assessment indicate the influence of the different X substituents on the conjugated π system.

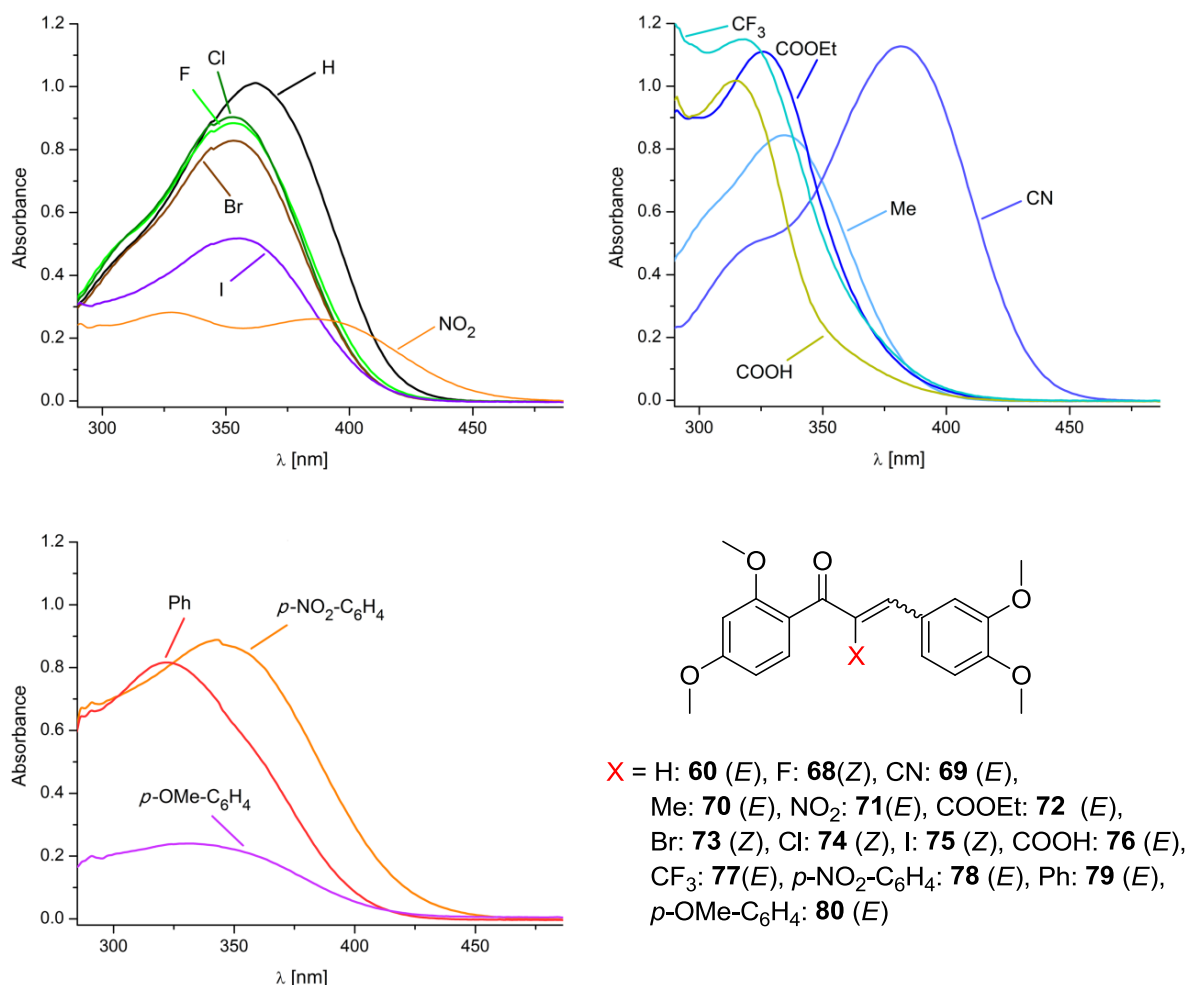


Figure 42: UV-Vis spectra of the α -X-TMCs in 100 mM TRIS-HCl pH 7.4, 2 mM EDTA/ethylene glycol (20:80) at 25 °C. All compounds were at 40 μM , only chalcones **71** and **77** were at 80 μM .

As in the case of α -H-chalcones, cysteamine (**107**) was used as the S-nucleophile to compare the α -X-TMCs and the applied reaction media is again 100 mM TRIS-HCl buffer pH 7.4, 2 mM EDTA/ethylene glycol (20:80). In the setting of the microtiter plate reader, this mixture enabled the kinetic measurements of α -X-TMCs within two to 63 h. Stopped-flow measurements were performed in the case of very fast reactions using the same solvent system in a similar way. Assay conditions with wavelengths, thiol concentrations and time intervals for α -X-TMCs are given in Table 7 and the results of the kinetic measurements are shown in Table 8. Representative k_2 value determinations for all tested α -X-TMCs-thiol pairs are given in the Appendix.

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Table 7: Wavelengths, fold thiol and time intervals (Δt) used in the kinetic assay of α -X-TMCs.

#	X	Wavelength [nm]	Chalcone [μ M]	Fold thiol ^a	Δt [s]
69	CN	380	40	12-60	Stopped-flow
71	NO ₂	390	80	60-108	Stopped-flow
77	CF ₃	355	80	60-108	Stopped-flow [*]
73	Br	360	40	100-500	Stopped-flow
74	Cl	335	40	12-60	18
78	<i>p</i> -NO ₂ -C ₆ H ₄	375	40	60-108	25
75	I	345	40	60-108	18
72	COOEt	320	40	60-108	25
68	F	350	40	100-500	90
80	<i>p</i> -OMe-C ₆ H ₄	350	40	60-108	600
70	Me	330	40	200-500	180
79	Ph	350	40	200-500	180
76	COOH	310	40	200-400	7 min
69	CN	380	40	60-108 ^b	Stopped-flow
71	NO ₂	390	80	60-108 ^b	Stopped-flow
77	CF ₃	355	80	20-40 ^b	11 [*]
73	Br	360	40	60-108 ^b	80
73	Br	360	40	60-108 ^c	80
73	Br	360	40	12-24 ^{d,e}	10 min

Fold thiol: **12-24**: 12, 15, 18, 21, 24; **12-60**: 12, 18, 24, 36, 48, 60; **20-40**: 20, 25, 30, 35, 40; **60-108**: 60, 72, 84, 96, 108; **100-500**: 100, 200, 300, 400, 500; **200-500**: 200, 300, 400, 450, 500; **200-400**: 200, 250, 300, 350, 400.

^a Cysteamine, ^b 2-mercaptoethanol, ^c 1,4-butanedithiol, ^d glutathione, ^e higher amounts than 27 fold of GSH gave decreasing instead of increasing k_{obs} values for the corresponding higher GSH concentrations. This could be because thiol oxidation may take place to get GSSG. ^{*} The data was fit to a single exponential + slope function to determine k_{obs} .

The great influence of a simple exchange of only one substituent on reactivity is revealed by the second-order-rate constants k_2 . The α -CN-TMC (**69**) has a k_2 value of $5750 \text{ M}^{-1} \text{ s}^{-1}$ which is about 1.6 million fold the k_2 value of α -COOH-TMC (**76**) ($0.00371 \text{ M}^{-1} \text{ s}^{-1}$). The CN substituent is a powerful electron-withdrawing group, which explains the highest reactivity of chalcone **69**. The NO₂ substituent has even a stronger electron-withdrawing ability, but unexpectedly a smaller k_2 value ($749 \text{ M}^{-1} \text{ s}^{-1}$) was determined for chalcone **71**. The structural differences between **69** and **71** (Figure 43) may account for the difference in their reactivity. The steric effects of the NO₂ group in chalcone **71** prefers the configuration where the NO₂ and the B-ring are not in the same side, whereas the slim size of the CN functionality of

chalcone **69** allows the CN group to be in the same side of the B-ring as shown in the X-ray structure of **69** (Figure 25). Hence, as indicated from both structures, the nucleophilic attack to the β -position of α -CN-TMC (**69**) is easier than that in the case of chalcone **71** with the steric demand of the NO₂ functionality.

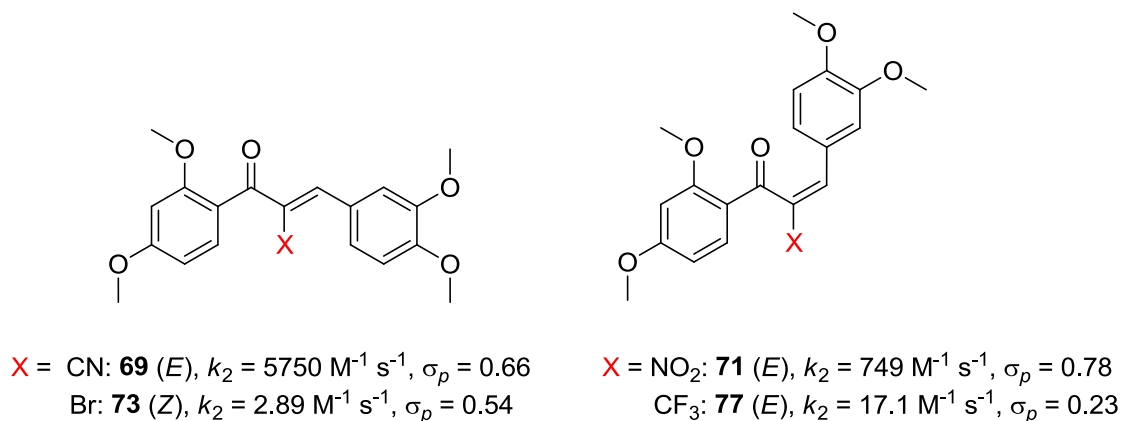
Table 8: Results of kinetic measurements of α -X-TMCs at 25 °C.*

107: R = CH₂CH₂-NH₂
109: R = (CH₂CH₂)₂-SH
111: R = CH₂CH₂-OH
112: Glutathione (GSH)

#	X	Thiol	$k_2^a / \text{M}^{-1}\text{s}^{-1}$	Rel. rate ^b
69	CN	107	5750 ± 130^c	1,600,000
71	NO ₂	107	749 ± 9.0^c	200,000
77	CF ₃	107	17.1 ± 1.8^c	4,600
73	Br	107	2.89 ± 0.080^c	780
74	Cl	107	1.65 ± 0.021	450
78	<i>p</i> -NO ₂ -C ₆ H ₄	107	0.293 ± 0.025	79
75	I	107	0.282 ± 0.015	76
72	COOEt	107	0.281 ± 0.029	76
60	H	107	0.193 ± 0.019	52
68	F	107	0.0168 ± 0.00035	4.5
80	<i>p</i> -OMe-C ₆ H ₄	107	0.00856 ± 0.0013	2.3
70	Me	107	0.00750 ± 0.00039	2.0
79	Ph	107	0.00669 ± 0.00029	1.8
76	COOH	107	0.00371 ± 0.000060	1.0
69	CN	111	368 ± 19^c	1/16 ^d
71	NO ₂	111	33.6 ± 0.34^c	1/22 ^e
77	CF ₃	111	1.49 ± 0.11	1/11 ^f
73	Br	111	0.135 ± 0.011	1/21 ^g
73	Br	109	0.151 ± 0.0086	1/19 ^g
73	Br	112	0.0399 ± 0.00050	1/72 ^g

^a Reactions were carried out in 100 mM TRIS-HCl pH 7.4, 2 mM EDTA/ethylene glycol (20:80) under pseudo-first-order conditions at concentrations of 40 μ M for all chalcones except chalcones **71** and **77** which were tested at 80 μ M. for chalcones **71**, **77** together with 12 to 500 fold cysteamine (**107**) or thiols **109**, **111** or **112**; ^b k_2 (X, **107**) relative to k_2 (COOH, **107**); ^c done by stopped-flow technique; ^d k_2 (CN, **111**) relative to k_2 (CN, **107**); ^e k_2 (NO₂, **111**) relative to k_2 (NO₂, **107**); ^f k_2 (CF₃, **111**) relative to k_2 (CF₃, **107**); ^g k_2 (Br, **111**), k_2 (Br, **109**) or k_2 (Br, **112**) relative to k_2 (Br, **107**). * The measurements and data analysis were done by Nafisah Al-Rifai and Sabine Amslinger.

This ability of the CF_3 to withdraw electrons is still high but much less than the CN and NO_2 , which is consistent with the order of reactivity of chalcone **77** after the CN **90** and NO_2 **95** chalcones with a k_2 value of $17.1 \text{ M}^{-1} \text{ s}^{-1}$.



reactivity order: $\text{CN} > \text{NO}_2 > \text{CF}_3 > \text{Br}$

Figure 43: Comparison of the structures, Hammett σ_p values and reactivities of the four most reactive chalcones in the α -X-TMCs series.

The halogens come next in the order of both reactivity and electron-withdrawing property. The α -Br-TMC (**73**) displayed the highest reactivity among the halogen derivatives with a k_2 value of $2.89 \text{ M}^{-1} \text{ s}^{-1}$. Upon comparing the CN chalcone (**69**) with the Br analogue (**73**) where both the α -X-substituent and the β -H are on opposite sides of the $\text{C}=\text{C}$, a good correlation can be found between the Hammett σ_p values (which indicate the inductive and resonance effects)⁹¹ and the k_2 values, *i.e.* the higher the σ_p value, the more reactive is the chalcone (Figure 43). This is also true when comparing the two chalcones that have the α -X-substituent and the β -H on the same side of the $\text{C}=\text{C}$, namely the more reactive NO_2 chalcone (**71**) with a σ_p value of 0.78 compared to the less reactive CF_3 chalcone (**77**) with a σ_p value of 0.23.

The Cl chalcone **74** showed a little bit less reactivity (k_2 : $1.65 \text{ M}^{-1} \text{ s}^{-1}$) than the Br one **73** which refers to the lower resonance effect of the Br substituent caused by the longer C-Br bond (1.905 \AA) compared to the C-Cl bond (1.736 \AA). The C-I bond (2.099 \AA) in α -I-TMC (**75**) is even longer and should lead to more activation, but the steric effect caused by the bulky size of the I atom (see X-ray structures in Figure 27) plays a significant role in decreasing the conjugation of the enone system and thus leads to less reactivity with a k_2 of $0.282 \text{ M}^{-1} \text{ s}^{-1}$ for the α -I-TMC (**75**). The lowest reactivity of the F analogue **68** is explained by

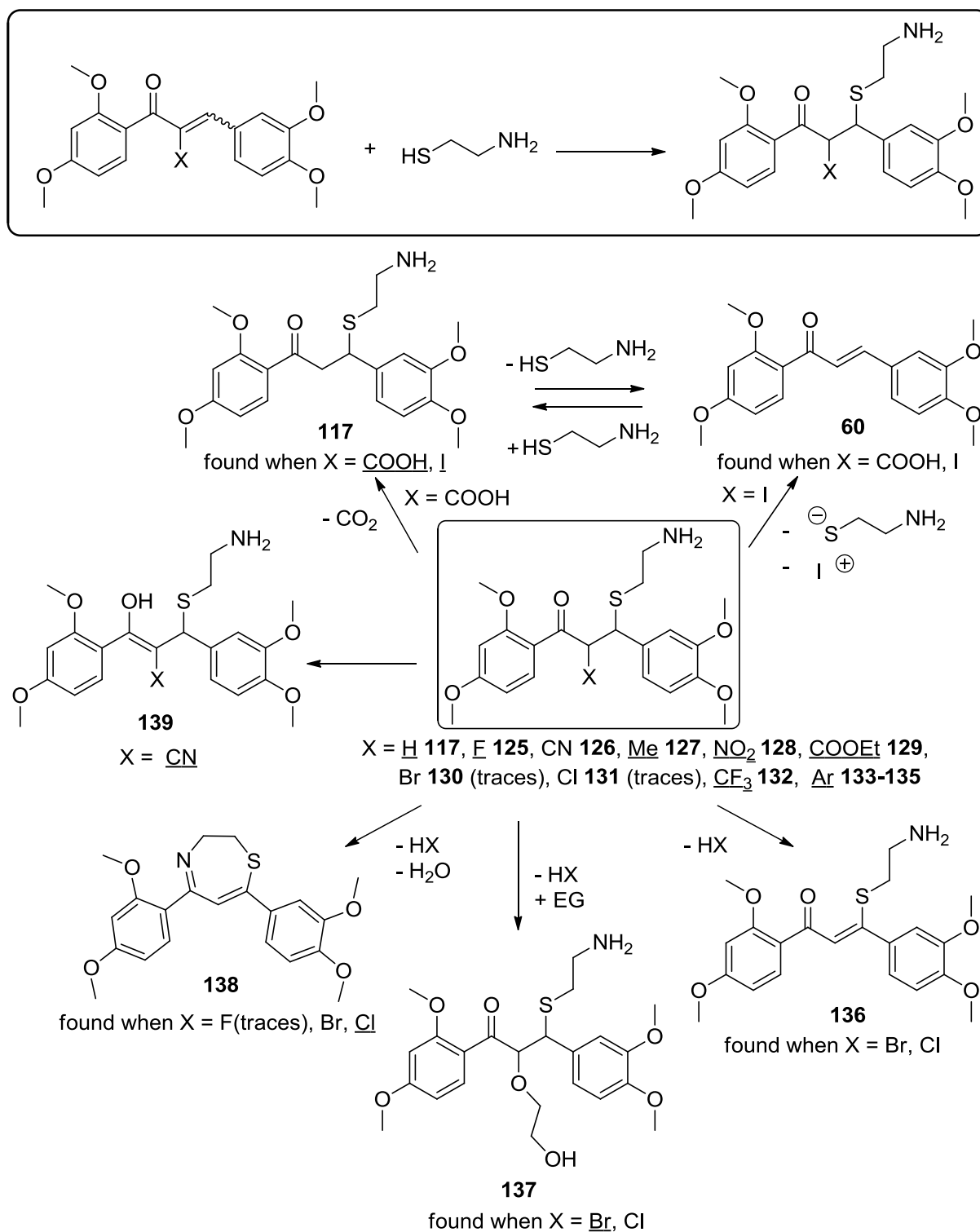
the high resonance effect of the small F atom which overrules its negative inductive effect making the F substituent the most deactivating halogen substituent (k_2 : $0.0168 \text{ M}^{-1} \text{ s}^{-1}$). The differences in the dihedral angles and C-X bond lengths indicated by the X-ray structures (Figures 24 and 27) of these four α -halogen-TMCs that adopt the *s-trans* conformation reveal that both electronic and steric effects have a great influence on the conjugation of the π system and thus on the reactivity, particularly in the case of α -I-TMC (**75**). This is of course taking into consideration that the preferred structure in solution (such as buffer/ethylene glycol mixture or in culture medium with serum proteins) might be different, but the relative spatial demand of the substituents which affects the conjugation of the π system should be comparable.

Regarding the α -carbon derivatives, the α -ester chalcone **72** showed higher reactivity (k_2 : $0.281 \text{ M}^{-1} \text{ s}^{-1}$) than the α -unsubstituted analogue **60** (k_2 : $0.193 \text{ M}^{-1} \text{ s}^{-1}$) which is attributed to the electron-withdrawing ability of the COOEt in **72**. On the other hand, the electron-donating property of the α -Me group is accounted for the very low reactivity of chalcone **70** (k_2 : $0.00750 \text{ M}^{-1} \text{ s}^{-1}$) compared to α -H-TMC (**60**). These electronic effects are also shown when comparing the α -Ph-TMC (**79**) with the α -aryl chalcone bearing the *p*-NO₂ functionality **78**. As expected, the activation of the electron-withdrawing NO₂ group is shown by the higher reactivity of chalcone **78** (k_2 : $0.293 \text{ M}^{-1} \text{ s}^{-1}$) compared to chalcone **79** (k_2 : $0.00669 \text{ M}^{-1} \text{ s}^{-1}$). The k_2 value of the *p*-OMe analogue **80** is $0.00856 \text{ M}^{-1} \text{ s}^{-1}$ which is lower than the *p*-NO₂ compound as it should be, but unexpectedly it is a little bit higher than that of the α -Ph-TMC (**79**). Differences in the *E/Z* equilibrium ratios or in the conformation may explain this behavior of the α -*p*-OMe chalcone **80**. The UV-Vis scans of the α -aromatic chalcones depicted in Figure 42 shows that α -*p*-OMe-C₆H₄-TMC (**80**) has a lower absorption than the two other α -aryl derivatives (**78** and **79**), which may give a sign regarding its distinct behavior.

The COOH group is the most deactivating substituent not only within the α -carbon series only but within the whole α -X-TMCs. The very low reactivity of chalcone **76** with a k_2 value of $0.00371 \text{ M}^{-1} \text{ s}^{-1}$ can be explained by deprotonation of the acid functionality at pH 7.4 producing the carboxylate where a negative charge is distributed between the two oxygen atoms and the carbon atom which is directly bonded with the α -position.

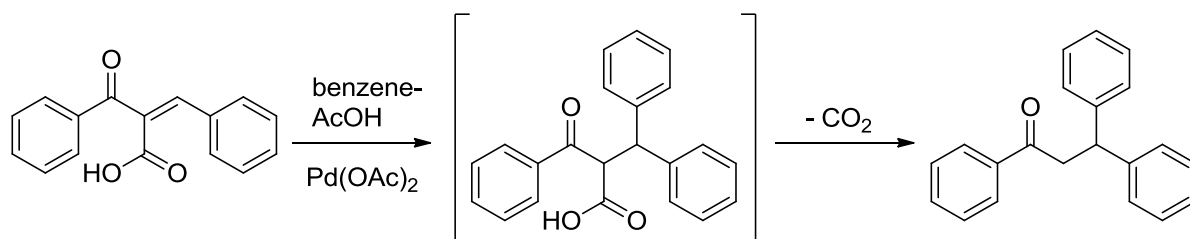
The UV-Vis scans of α -CN-TMC (**69**) with different thiols (Figure 32) showed that this chalcone can differentiate between thiols of different reactivities. To have more proof for such a differentiation which is important in biological systems especially for potential selectivity, kinetic measurements were performed with other thiols than cysteamine (**107**). 1,4-Butanedithiol (**109**), 2-mercaptoethanol (**111**) and glutathione (GSH, **112**) were chosen for this aim. The four most reactive α -X-TMCs (X = CN (**69**), NO₂ (**71**), CF₃ (**77**), Br (**73**)) were tested with 2-mercaptoethanol (**111**) which is less reactive than cysteamine (**107**) and interestingly the order of reactivity was the same as that obtained upon reaction with **107** as indicated by the k_2 values of 368 M⁻¹ s⁻¹ for α -CN-TMC (**69**), 33.6 M⁻¹ s⁻¹ for α -NO₂-TMC (**71**), 1.49 M⁻¹ s⁻¹ for α -CF₃-TMC (**77**) and 0.135 M⁻¹ s⁻¹ for α -Br-TMC (**73**). Chalcone **73** was also tested with thiol **109** and **112** to give k_2 value of 0.151 M⁻¹ s⁻¹ and 0.0399 M⁻¹ s⁻¹, respectively. The lower reactivity of thiols **109**, **111**, **112** with the Br chalcone **73** compared to cysteamine (**107**) indicates again their lower nucleophilicity as shown before in the case of the CN chalcone **69** (Figure 32).

The LC-MS analyses on the crude reaction mixtures of α -X-TMCs with cysteamine proved the formation of the expected Michael addition products (**117** and **125-135**, Scheme 43) in all cases except for the I- and COOH-chalcones **75** and **76**, respectively. Here only downstream products were detected. More specifically, α -COOH-TMC (**76**) gives compound **117** upon decarboxylation of its Michael adduct followed by a retro-Michael addition to form chalcone **60** which was also produced in the case of chalcone **75** by the loss of the thiolate and iodine atom through formation of an iodonium ion.



Scheme 43: The initial step of the reaction cysteamine (**107**) with α -X-TMCs in 100 mM TRIS-HCl pH 7.4, 2 mM EDTA/ethylene glycol (20:80) and the subsequent reactions of the initial thia-Michael products. The products were confirmed by (LC-MS) analysis. The major products are marked by an underlined substituent X. EG = ethylene glycol.

A similar decarboxylation was reported by Yamamura from his α -COOH benzene adduct of α -acid chalcone (Scheme 44) although under different conditions.²⁷



Scheme 44: The decarboxylation of α -COOH benzene adduct reported by Yamamura.²⁷

For α -Br-TMC (**73**) and α -Cl-TMC (**74**), the expected thia-Michael adducts **130** and **131** respectively were detected but just in trace amounts and just when the measurements were done directly after mixing the chalcone solution with the thiol **107** solution. However, the formation of subsequent products was again observed. Firstly compound **136** was formed due to leaving ability of Cl and Br substituents. Then addition of the solvent (*i.e.* ethylene glycol) to the α -position of **136** gave compound **137**. The seven-membered intramolecular condensation product, *i.e.* thiazepine **138** found in the case of Br- and Cl-TMCs (**73** and **74** respectively) was formed by the nucleophilic attack of the NH_2 residue of compound **136** on its carbonyl group.

Overall, the downstream products for in the case of Br, Cl and COOH chalcones **73**, **74**, **76** respectively prove the leaving group quality of these substituents, as Br^- , Cl^- and CO_2 . The iodine atom in chalcone **75** is leaving as I^+ in a retro electrophilic addition to the double bond of the enone system. The chromatograms of LC-MS analyses of all the tested α -X-TMCs are shown in the Appendix.

In the case of α -CN-TMC (**69**), the LC-MS measurements showed only trace amounts of the expected Michael adduct in addition to the starting chalcone **69**. This could be rationalized by the elevated temperature used for the HPLC separation which is 40 °C. It was reported that the Michael addition to α -CN compounds is reversible at high temperatures (50 °C).⁹² To prove this explanation, the LC-MS experiment was repeated at 25 °C. There, the normal addition product was observed as the major product. This result was also confirmed by ^1H NMR measurements of chalcone **69** in presence of cysteamine (**107**) at 25 °C, 50 °C and then again at 25 °C (Figure 44). The spectra (Figure 44e and b-d) shows a mixture of *E/Z* isomers of the enol form of the thia-Michael adduct **139** with OH signals at $\delta = 11.39$ and 11.17 ppm and singlets for the hydrogens of the β -carbon ($\text{H}_\beta^{\text{'''}}$) at $\delta = 5.37$ and 4.24 ppm (Figures 44e and b-d). Figure 44c shows the partial reversibility of the Michael addition at 50 °C, which is

consistent with finding just trace amounts of the addition adduct in the case of the CN chalcone **69** during the LC-MS measurement.

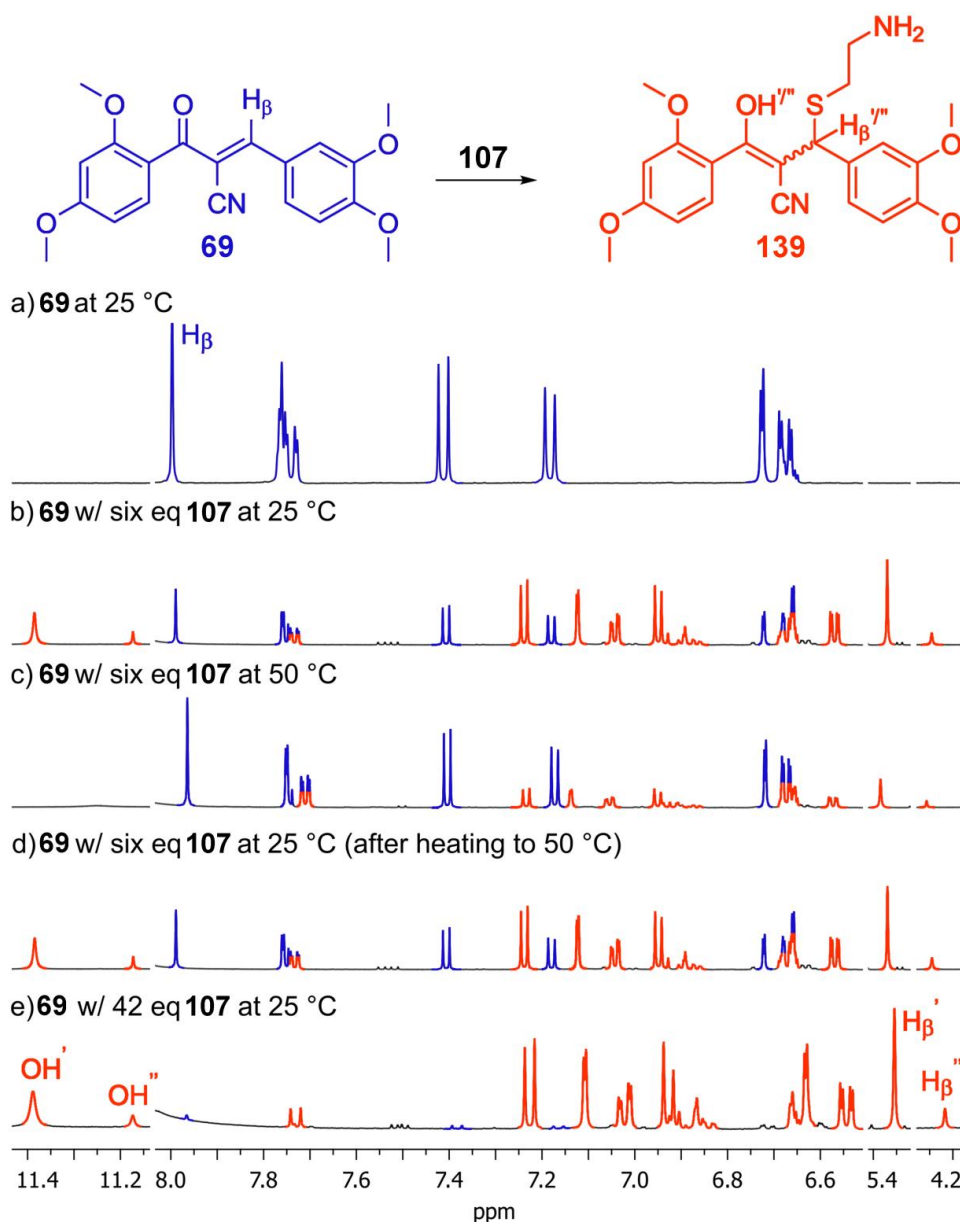
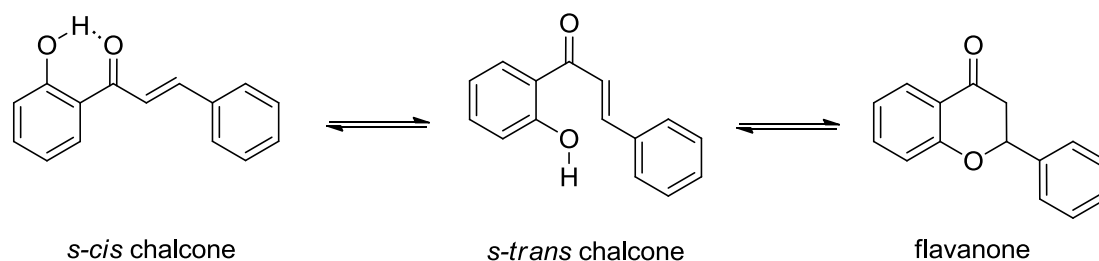


Figure 44: 1H NMR spectra of α -CN-TMC (**69**) with cysteamine (**107**) in DMSO- d_6 .

3.2.6. Analysis of reactivity of α -X-HCs

It was firstly tried to determine the k_2 of the reaction of α -CN-HC (**95**) with cysteamine (**107**) by the developed UV-Vis based kinetic thiol assay, but it was not possible to get clean kinetics. In addition it was noticed that upon dissolving in the solvent mixture of the kinetic assay the yellow color of chalcone **95** suddenly disappeared. This behavior could be explained by the cyclization of the yellow chalcone **95** to the corresponding colorless flavanone. The

cyclization of the 2'-OH chalcones to flavanones (Scheme 45) is a common phenomenon. It has been reported to occur mainly in protic solvents and it is affected by pH, temperature, α -substituents and substitution pattern on the chalcone rings.⁹³⁻¹⁰⁰ In 2013, this isomerization was also investigated under cell culture conditions.¹⁰¹



Scheme 45: The isomerization between the chalcone and the flavanone.

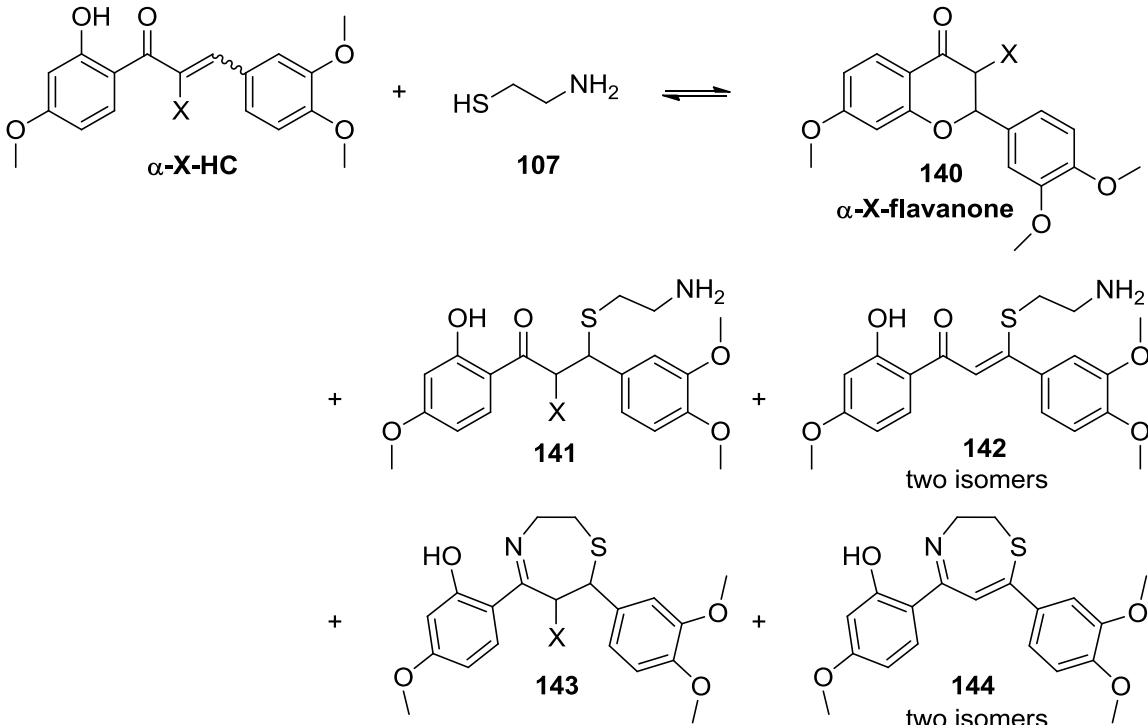
To prove these explanations and to study the tendency of the prepared α -X-HCs to isomerize giving the cyclized form (flavanone) especially since most of them adopt the *s-trans* conformation, LC-MS analysis was performed. The LC-MS samples were prepared under the kinetic assay conditions, *i.e.* 40 μ M chalcone in 100 mM TRIS-HCl buffer pH 7.4, 2 mM EDTA/ethylene glycol (20:80) without and with cysteamine (**107**). Based on the DAD chromatograms a relative quantification was done (Table 9) just to have an idea on what is going on.

The flavanone structures were identified by their UV-Vis spectra obtained in the LC-MS measurements, since both the chalcone form and its cyclized form have the same MS. Three points should be taken into account to assign the peaks of the obtained chromatograms correctly:

1. The λ_{\max} of the HCs which is reported to be in the range of 350-450 nm.⁹³ The λ_{\max} of the synthesized α -X-HCs is in 340-380 nm range for X= H (**61**), F (**94**), CN (**95**), Cl (**98**), *p*-NO₂-C₆H₄ (**100**) and in 290-300 nm for X= Br (**97**) and CF₃ (**99**).
2. The retention time of the peaks, since it has been reported that the chalcones have higher retention time than the corresponding flavanones.¹⁰²
3. The MS-fragment that formed upon elimination of HX which should be more intense in the flavanone case since such elimination is easy from the flavanone to form the corresponding flavones.

RESULTS AND DISCUSSION

Table 9: Results of LC-MS studies of α -X-HCs without and with 60 fold cysteamine (**107**).^a

									
#	X	Eq ^b 107	compounds detected by LC-MS						
			α -X-HC	140	141	142	143	144	Other ^c
61	H	0	> 95%	trace	-	-	-	-	-
61	H	60	+	-	+++	-	+	-	-
94	F	0	> 95%	trace	-	-	-	-	-
94	F	60	+++	trace	++	-	trace	-	-
95	CN ^{d,e}	0	++	+++	-	-	-	-	-
95	CN	60	-	-	++	-	-	-	++
96	Me	0	> 95%	trace	-	-	-	-	-
96	Me	60	95%	trace	trace	-	-	-	-
97	Br	0	+	+++	-	-	-	-	+
97	Br	60	trace	+	-	trace	-	++	+
98	Cl	0	++	+++	-	-	-	-	-
98	Cl	60	trace	+	-	+	-	+++	^f
99	CF ₃ ^d	0	+	++	-	-	-	-	+
99	CF ₃ ^d	60	+	++	-	-	-	-	-
100	<i>p</i> -NO ₂ -C ₆ H ₄	0	+	+++	-	-	-	-	-
100	<i>p</i> -NO ₂ -C ₆ H ₄	60	+	+++	+	-	-	-	-

^a Reactions were carried out in 100 mM TRIS-HCl pH 7.4, 2 mM EDTA/ethylene glycol (20:80 B/EG) with 40 μ M of α -X-HCs. The incubation time prior injection was 1-2 h and the HPLC analysis performed at 40 °C;

^b equivalents used of cysteamine (**107**); ^c see chromatograms in the Appendix; ^d measured directly after mixing in B:EG; ^e measured at 25 °C HPLC column temperature; ^f see Figure 46. Molecular ratios were estimated by UV measurements and relative amounts were assigned as follows: +++: < 95-60%; ++: < 60-30%; +: < 30-5; trace: < 5%.

As indicated in Table 9, in the absence of cysteamine (**107**) only trace amounts of the cyclized form **140** were detected in the case of α -H-HC (**61**), α -F-HC (**94**) and α -Me-HC (**95**). On the other hand, the flavanone was the predominant component in all the other α -X-HCs (X = CN (**95**), Br (**97**), Cl (**98**), CF₃ (**99**) and *p*-NO₂-C₆H₄ (**100**)), which indicates the activation of the β -position caused by these α -substituents. As an example, Figure 45 shows the results of LC-MS analysis of α -Cl-HC (**98**) without thiol **107** after about 1:45 h incubation prior to the LC-MS measurements. The graphs of all the other α -X-HCs without and with cysteamine are given in the Appendix.

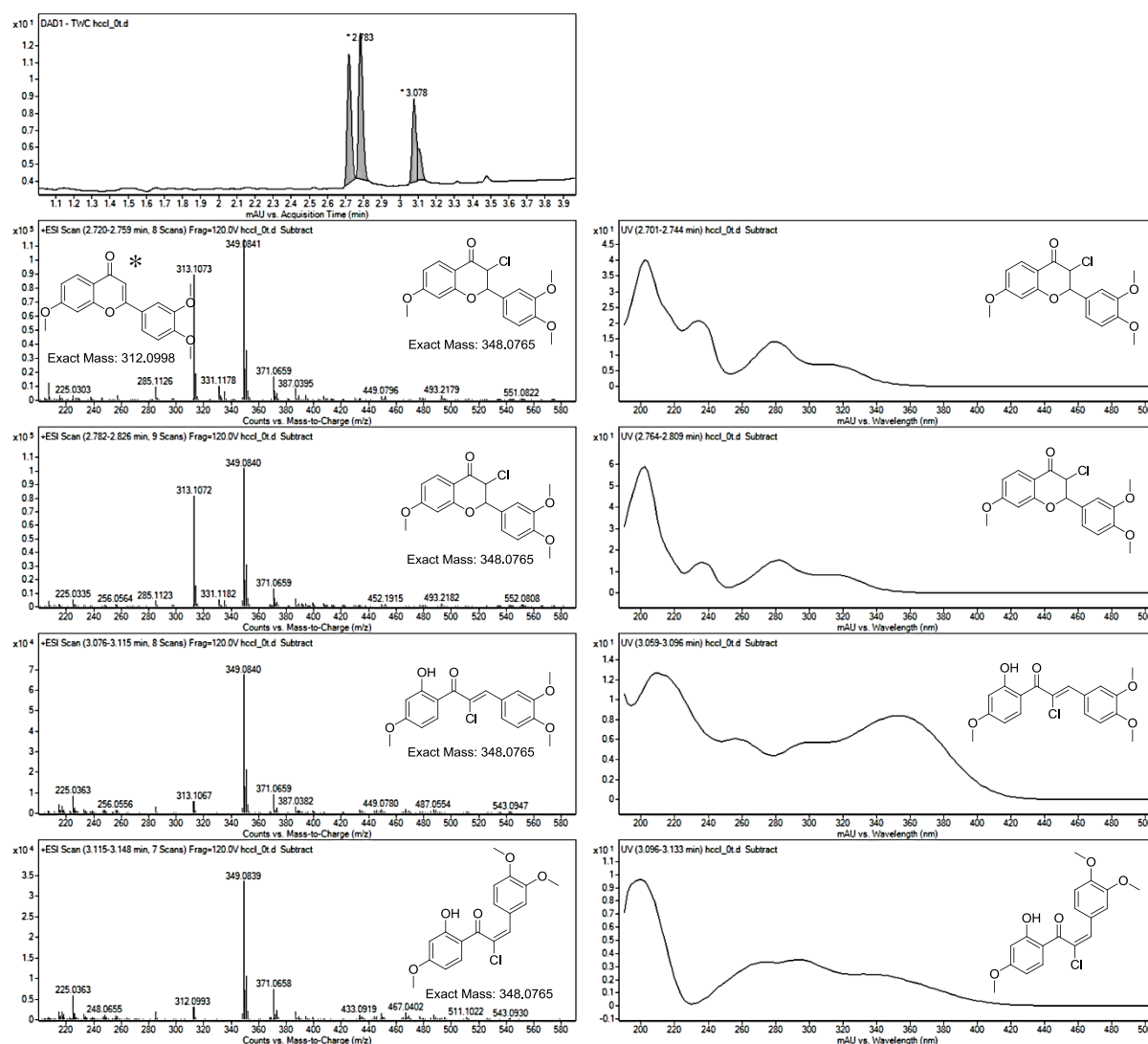


Figure 45: LC-MS analysis of α -Cl-HC (**98**) dissolved in 100 mM TRIS-HCl buffer pH 7.4, 2 mM EDTA/ethylene glycol (20:80); the incubation time was about 1:45 h. The top graph shows the HPLC chromatogram with DAD detection (relative quantification). For particular retention times (peaks) the UV scans are displayed (right side) and the ESI (+ mode) MS spectrum (left side). * Fragment by HCl elimination.

The top graph in Figure 45 shows the HPLC chromatogram of α -Cl-HC (**98**) with DAD detection. Despite the fact that the pure *Z*-isomer of **98** was initially used, two isomers were detected in the chalcone form of **98** at 3.0 and 3.1 min. Additionally, two other isomers were found but with lower retention times (2.7 and 2.8 min) for the cyclized form of α -Cl-HC (**98**). This indicates that there is an equilibrium between the two forms. The first two UV spectra which correspond to the flavanone form indicate the absence of the chalcone absorption maximum at about 360 nm, which confirms that they are not the yellow chalcone anymore although they have the same exact mass. What is noteworthy is the ‘minus HCl’-fragment with $[M-HCl+H]^+$ of 313.1072 which is more intense in the cases of flavanone than in the chalcone. These findings prove that the assignment of peaks at lower retention time is correct.

Upon reaction of α -Cl-HC (**98**) with 60 fold cysteamine (**107**), no normal addition product **141** was found (Figure 46).^{*} Instead, four other products were detected, most are similar to those produced by the Cl-TMC analogue **98** (Scheme 43). These products are: two isomers of 2,3-dihydro-1,4-thiazepine **144** as the major product, two isomers of the Michael adduct without HCl **142**, a cyclic cysteamine adduct with a mass of 389.1297 which was formed by intramolecular S_N2 replacement of Cl by the amine functionality of cysteamine and finally a compound (exact mass: 223.0667) that was formed upon reaction of cysteamine with the aldehyde produced from a retro-Aldol reaction. Moreover, traces of the starting material α -Cl-HC (**98**) both in the chalcone and in the flavanone form could be detected.

The behavior of α -Br-HC (**97**) was similar to the Cl derivative **98** in producing many subsequent products. Beside the respective chalcone and the flavanone form, α -CF₃-HC (**99**) showed three unidentified products in presence of cysteamine (**107**), two of them were also detected in the absence of cysteamine (**107**).

^{*} The measurement was done after about 1:45 hr of incubation.

RESULTS AND DISCUSSION

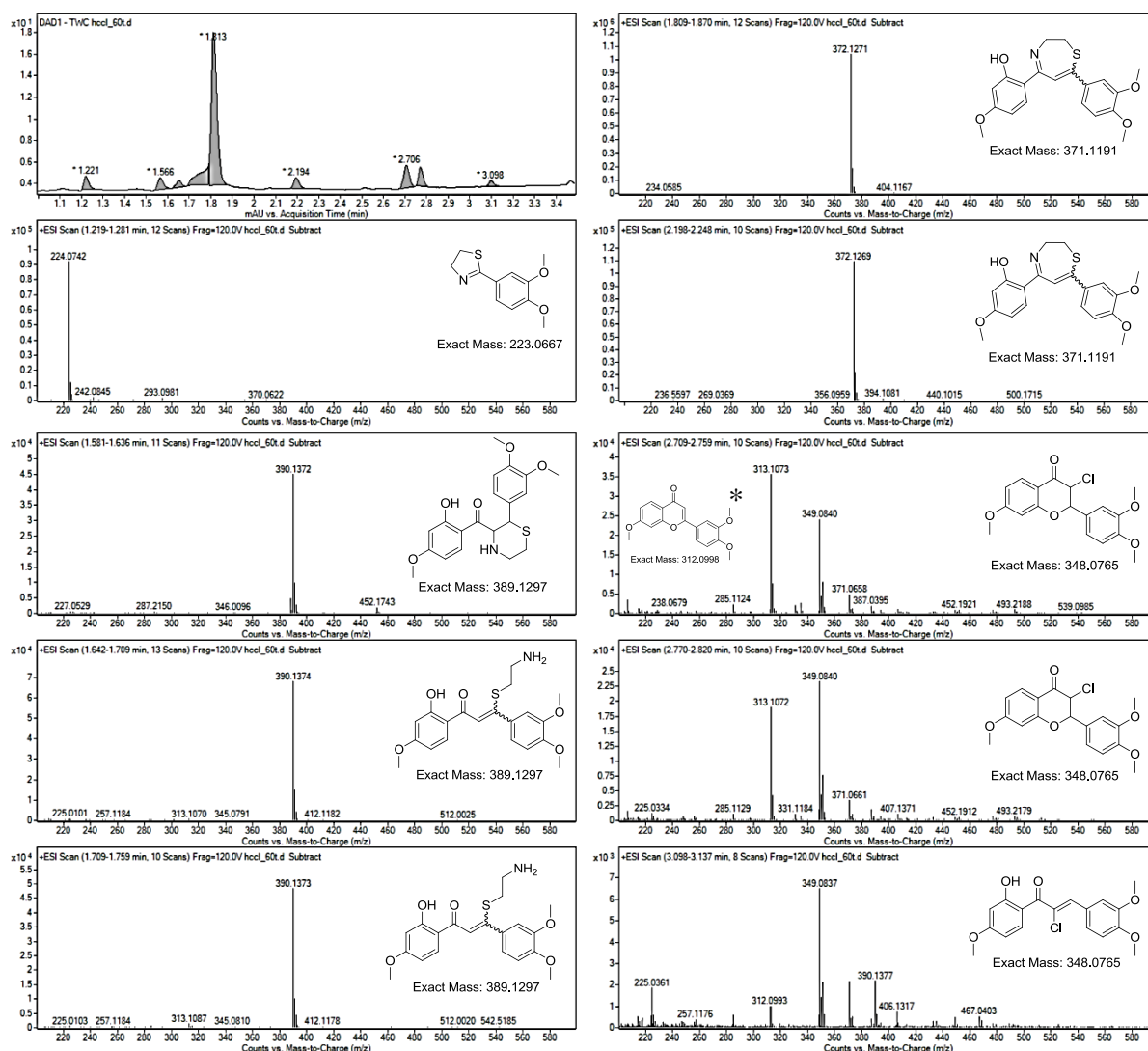
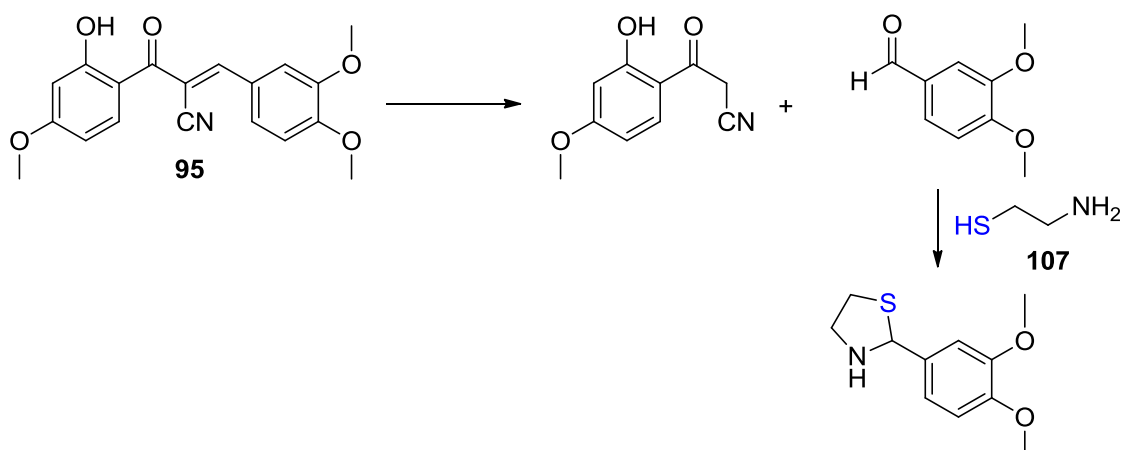


Figure 46: LC-MS analysis of α -Cl-HC (**98**) dissolved in 100 mM TRIS-HCl buffer pH 7.4, 2 mM EDTA/ethylene glycol (20:80) in presence of 60 fold cysteamine (**107**), the incubation time was about 1:45 h. The top graph shows the HPLC chromatogram with DAD detection (relative quantification). For particular retention times (peaks) the UV scans are displayed (right side) and the ESI (+ mode) MS spectrum (left side). * Fragment by HCl elimination.

In presence of 60 fold cysteamine (**107**), the α -H-HC (**61**) and α -F-HC (**94**) gave the expected addition product **141** in good quantities, while only traces of such a product was found in the case of the Me analogue **96** (in the tested time frame of about 2 h) due to its lower electrophilicity compared to **61** and **94** (Table 9). In the case of the α -aryl-HC (**100**), the addition product **141** was also the only product found but not in a big amount. α -CN-HC (**95**) gave unusual products upon reaction with cysteamine (Scheme 46) which are produced by a retro-Aldol condensation followed by reaction of the produced aldehyde with cysteamine (**107**).



Scheme 45: The formation of the products which were detected by LC-MS for α -CN-HC (**95**) in presence of cysteamine (**107**).

So, the CN-, Cl-, Br-, CF₃- and *p*-NO₂-C₆H₄-substituents have been proven to enhance the electrophilicity of the β -position of the chalcone as they did in the case of α -X-TMCs, but here this enhancement promotes the conversion of the chalcones into α -X-flavanones. Whereas, the H and the deactivating groups (F, Me) were not able to induce the cyclization of α -X-HCs to a reasonable extent. Since the cyclization is not favored in the case of α -F-HC (**94**), it was possible to determine its k_2 value upon reaction with cysteamine (**107**). The reactivity enhancement gained by the 2'-OH led to a k_2 value* of $0.0347 \pm 0.0029 \text{ M}^{-1} \text{ s}^{-1}$ for chalcone **94** which is about 2 fold the k_2 value of α -F-TMC (**68**).

3.3. Biological tests for the synthesized chalcones

3.3.1. Influence of α -X-TMCs on the cell viability, the HO-1 upregulation and the NO production¹⁰³⁻¹⁰⁴

Hannelore R cker performed during her PhD work in vitro assays of the murine macrophage cell line RAW264.7 with the synthesized α -X-TMCs and could show that their chemical reactivity which was revealed by the kinetic assay correlates very well with their biological activity.

Firstly, she did cell viability assays in a concentration range of 1 to 25 μM and found no toxicity in this range for the chemically most reactive chalcones, namely α -CN-TMC (**69**) and α -NO₂-TMC (**71**), whereas as the third one in the chemical reactivity order (α -CF₃-TMC (**77**)) was very toxic being the most toxic compound in the series of α -X-TMCs. The toxicity limits

* Measured by Hermina Petkes.

of α -X-TMCs are given in Table 10. Excluding chalcones **69** and **71**, a good correlation was found between the cytotoxicity of the chalcones (with X = CF₃ (**77**), Br (**73**), Cl (**74**), I (**75**)) and their chemical reactivity (k_2 values), *i.e.* the higher the k_2 value the more toxic is the chalcone.

Secondly, Hannelore R cker and Dita Fritsch made Western blots to show the influence of α -X-TMCs on the Nrf2-dependent anti-inflammatory protein heme oxygenase-1 (HO-1). At 1 μ M (0.5 μ M for chalcone **77** due to its higher toxicity) the α -X-TMCs **77**, **73**, **74** and **78** were active as HO-1 inducers again in the same order of their chemical reactivity.

Finally, Hannelore R cker performed Griess assay for the α -X-TMCs to determine their ability to inhibit NO. As illustrated in the introduction section, the expression of iNOS and its activity is connected with the transcription factor NF- κ B (Figure 4), which implies that the inhibition of the NO production can be used as an indication of anti-inflammatory activity.

Table 10: Results of the in vitro toxicity assay and NO assay of α -X-TMCs with RAW264.7 cells.^{103 104}

#	X	Toxicity limit ^{a,b} [μ M]	Toxicity limit in presence of LPS ^a [μ M]	Inhibition of NO production % ^c ([μ M])
69	CN	25	25	16.4 \pm 8.7 (1)
71	NO ₂	25	10	1.7 \pm 8.5 (1)
77	CF ₃	0.5	0.5	84.6 \pm 8.9 (0.5)
73	Br	1	1	67.4 \pm 11.7 (1)
74	Cl	5	1	47.2 \pm 14.6 (1)
78	<i>p</i> -NO ₂ -C ₆ H ₄	25	10	1.1 \pm 9.8 (1)
75	I	5	5	22.0 \pm 12.4 (1)
72	COOEt	10	10	46.0 \pm 9.4 (5)
60	H	10	10	60.9 \pm 18.8 (5)
68	F	25	25	29.6 \pm 20.2 (5)
80	<i>p</i> -OMe-C ₆ H ₄	25	10	-2.2 \pm 4.3 (5)
70	Me	25	10	-34.7 \pm 15.2 (5)
79	Ph	25	25	9.7 \pm 12.9 (5)
76	COOH	25	25	-0.7 \pm 12.2 (5)

^a Determined by MTT assay. ^b no LPS was added. ^c Determined by Griess assay with LPS-stimulated (10 ng mL⁻¹) cells. Chalcones are ordered according to their chemical reactivity. Measurements were done by Hannelore R cker.

The chemically active compounds were tested at 1 μM (0.5 μM for **77**) and the others at 5 μM . The most active chalcone was $\alpha\text{-CF}_3\text{-TMC}$ (**77**) which gave about 85% reduction of the NO production at 0.5 μM . Chalcones $\alpha\text{-Br-TMC}$ (**73**), $\alpha\text{-Cl-TMC}$ (**74**), $\alpha\text{-I-TMC}$ (**75**) displayed also good activity at 1 μM . The activity of these four compounds is also in the order of their chemical reactivity: **77** > **73** > **74** > **75**. The other $\alpha\text{-X-TMCs}$ did not show significant activity at 1 μM and even at 5 μM only chalcones **60**, **68**, **72** were active (Table 10).

It is noteworthy that despite the high chemical reactivity of the $\alpha\text{-CN}$ **69** and $\alpha\text{-NO}_2$ **71** derivatives, they were not biologically active – both in the HO-1 induction and the reduction of the NO production. This can be referred to their extremely high electrophilicity which makes them targets for the cellular electrophile trap GSH.¹⁰⁵ So it is important to find a certain reactivity window to retain activity. The reactivity that was determined by k_2 values of 2-20 $\text{M}^{-1} \text{s}^{-1}$ led to high potency, but a good potency was found down to k_2 values of 0.2 $\text{M}^{-1} \text{s}^{-1}$.

In conclusion, these results indicate that a prominent modulation in both chemical reactivity and biological activity of TMCs was achieved merely by a single modification. The chemical reactivity-biological activity relationship for $\alpha\text{-X-TMCs}$ can be stated as follows:

[Michael acceptor activity ($\alpha\text{-X-TMCs}$)] \propto [Inhibition of NO production ($\alpha\text{-X-TMCs}$)]

for $X = \text{H}, \text{F}, \text{Cl}, \text{Br}, \text{I}, \text{CF}_3$;

Chemical reactivity-biological activity order: $\text{CF}_3 > \text{Br} > \text{Cl} > \text{I} > \text{H} > \text{F}$.

3.3.2. Influence of $\alpha\text{-X-pHCs}$ and $\alpha\text{-X-HCs}$ on the cell viability and the NO production

2'-O-Isopropyl (protected) chalcones $\alpha\text{-F-pHC}$ (**104**) and $\alpha\text{-CN-pHC}$ (**105**)

MTT test

In contrast to $\alpha\text{-F-TMC}$ (**68**)¹⁰³⁻¹⁰⁴ which was not toxic at 1-25 μM , $\alpha\text{-F-pHC}$ (**104**) reduced the cell viability by about 20% at 10-25 μM . This difference can be referred to structural differences shown by their X-ray analysis. The B-ring of **104** (Figure 24) is rotated to be in a different situation from that in **68** (Figure 29) which may affect the activity of **104** compared to **68**. No toxicity difference was found between $\alpha\text{-CN-pHC}$ (**105**) and $\alpha\text{-CN-TMC}$ (**69**) in the range of 1-25 μM . Chalcones **104** and **105** were also tested at higher concentrations, **104** showed a reduction of 60% in cell viability at 50-100 μM , while **105** that was tested up to 75 μM reduced the cell viability by 29% at this concentration. Figure 47 (A and B) show the

results on MTT assays of **68** and **104** without LPS (which was discussed above) and with LPS (which was done to be sure about the cell viability in presence of LPS which is needed for the NO assay).

NO (Griess) assay

α -F-pHC (**104**) was tested for its ability to inhibit the production of NO at 5 μ M as it was toxic at higher concentrations. At this concentration it showed 17% inhibition of the NO production (Figure 47C), while its TMC analogue **68**¹⁰³⁻¹⁰⁴ inhibited the production of NO by 30% at this concentration. At 50 μ M α -CN-pHC (**105**) gave an inhibition of 36% (Figure 47C). No inhibition was found at 1 μ M while its TMC analogue **69** showed a 16% inhibition at the same concentration. The lower activity of **105** is consistent with its lower k_2 value ($3060 \pm 58 \text{ M}^{-1} \text{ s}^{-1}$) upon reaction with cysteamine compared to that of **69** ($5750 \text{ M}^{-1} \text{ s}^{-1}$). This implies less electrophilicity for **105** as a result of the reduced conjugation of the π system which can be attributed to the steric influence of the isopropyl group in **105**.

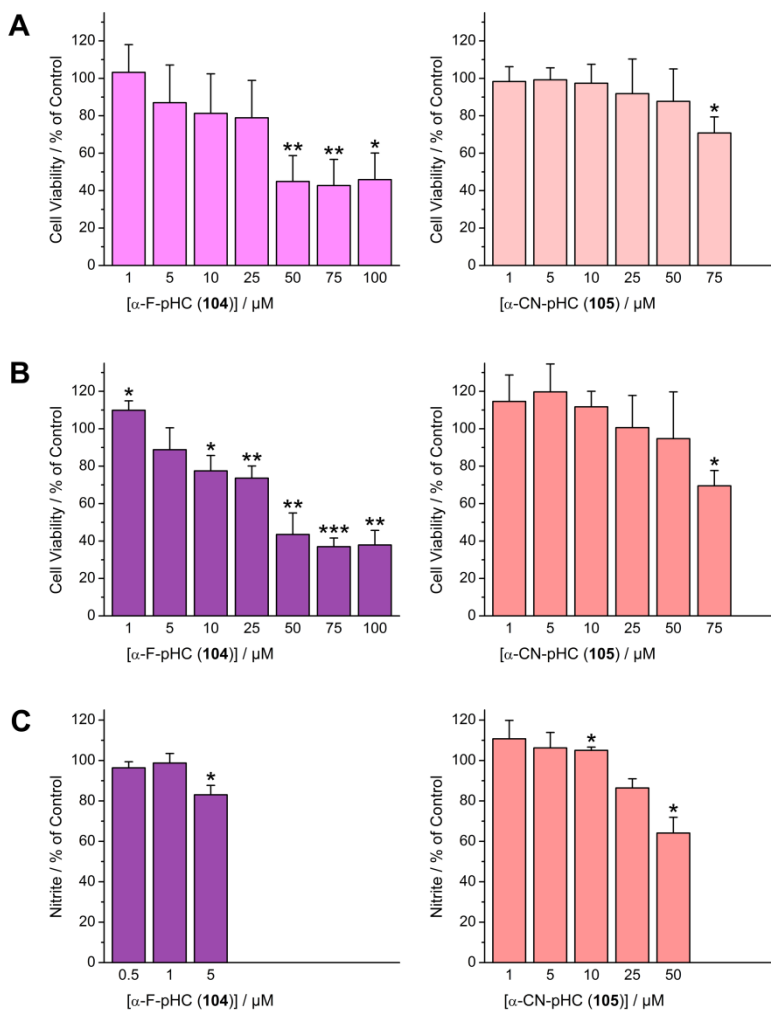


Figure 47: Results of in vitro tests of α -X-pHCs **104** and **105** with RAW264.7 cells after an incubation time of 24 h. **A)** MTT test (no LPS was added), **B)** MTT tests in presence of $10 \text{ ng}\cdot\text{mL}^{-1}$ LPS, **C)** Griess assays in presence of $10 \text{ ng}\cdot\text{mL}^{-1}$ LPS. Data represent at least three independent experiments performed in quadruplicates. Levels of significance: ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$.

α -X-HCs (X = H (**61**), F (**94**), CN (**95**), Me (**96**), Br (**97**), Cl (**98**), CF_3 (**99**), $p\text{-NO}_2\text{-C}_6\text{H}_4$ (**100**))*

MTT test

The α -unsubstituted chalcone **61** has a toxicity limit of $25 \mu\text{M}$ where it reduced the cell viability only by 18%, whereas its TMC analogue **60** was toxic at this concentration with a 54% reduction of the cell viability.¹⁰³⁻¹⁰⁴ A significant toxicity was found for α -F-HC (**94**) at $25 \mu\text{M}$ up to $100 \mu\text{M}$ (Figure 48). At $25 \mu\text{M}$ it reduced the cell viability by 43%, while α -F-TMC **68** did not affect the cell viability at this concentration (Table 10). This reflects the enhanced electrophilicity of the HC **94** compared to the TMC **68**. At $10 \mu\text{M}$, 17% reduction in cell viability was found for chalcone **94** (Figure 48). In presence of LPS, the reduction was

* The α -H-HC (**61**) was tested by Hannelore Rucker. All the other α -X-HCs were tested by Nafisah Al-Rifai.

elevated to 28% at the same concentration (Figure 49), therefore this concentration was excluded from the Griess assay of **94**. α -CN-TMC (**69**) was not toxic at 1-25 μ M (Table 10), and so is α -CN-HC (**95**) that has no significant effect on the cell viability up to 50 μ M. It was not possible to do the MTT assay for **95** at higher concentrations due to its insolubility. Regarding the α -Me-HC (**96**), a significant toxicity was found at the highest concentration (100 μ M) with a 36% reduction in cell viability (Figure 48), whereas in presence of LPS (Figure 49) the cell viability was reduced at 75 μ M so this concentration was not taken in the following NO test. No toxicity was reported for α -Me-TMC (**70**) up to 25 μ M.

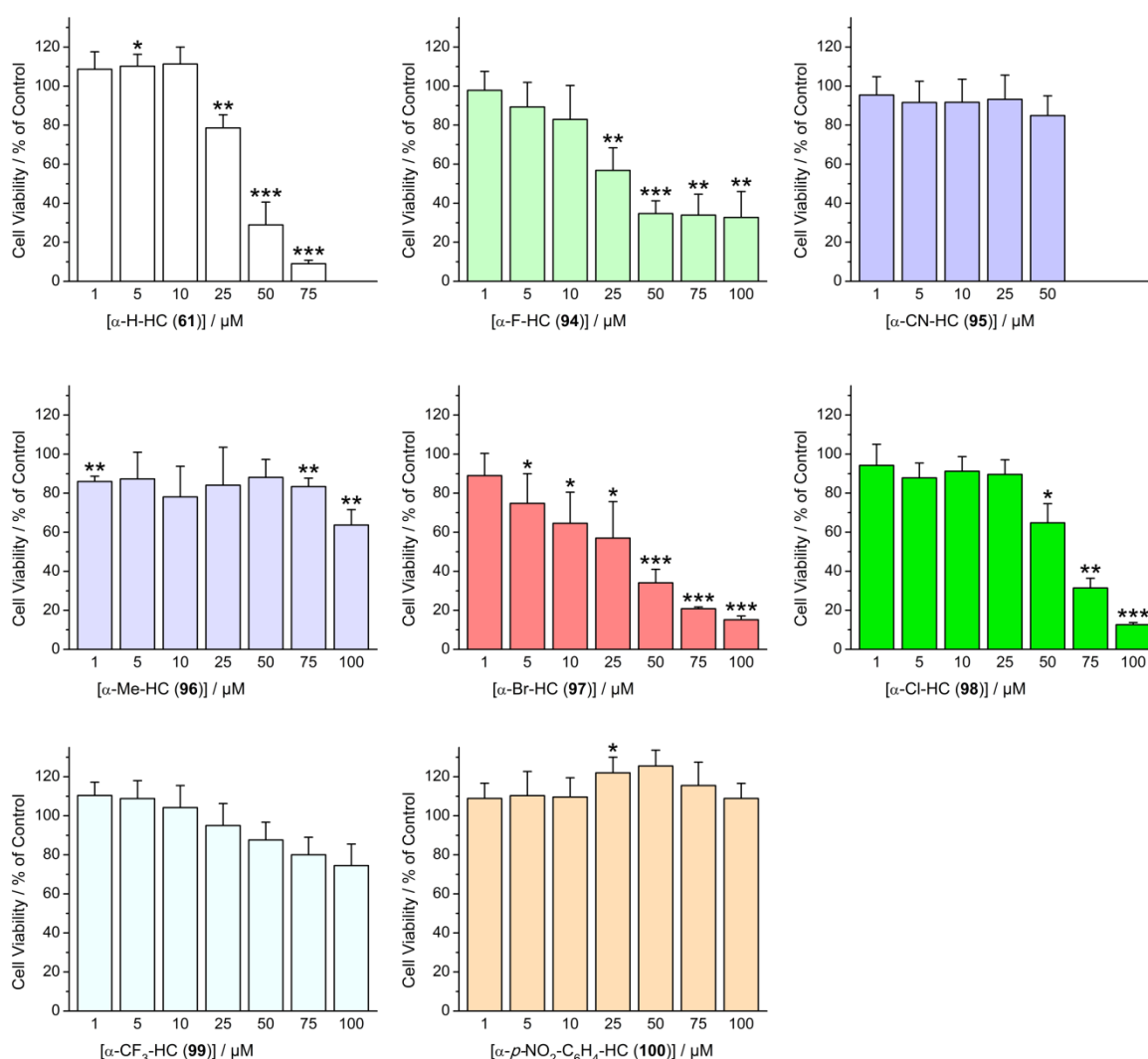


Figure 48: Results of in vitro cell viability assays of α -X-HCs **61** and **94-100** with RAW264.7 cells for 24 h (no LPS was added). Data represent at least three independent experiments performed in quadruplicates. Levels of significance: ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$. The testing of **61** was done by Hannelore R cker, while the other chalcones were tested by Nafisah Al-Rifai.

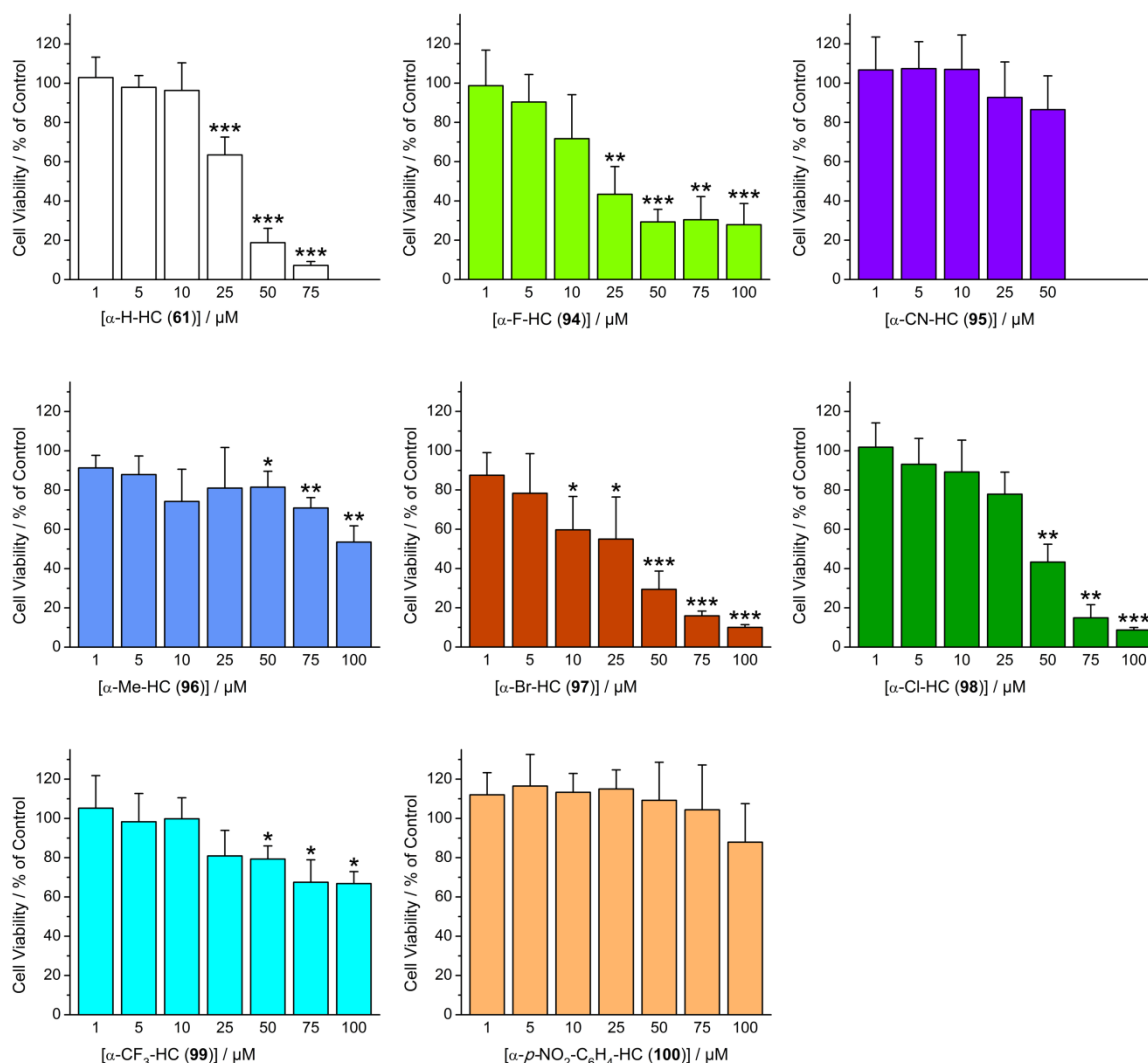


Figure 49: Results of in vitro cell viability assays of α -X-HCs **61** and **94-100** with RAW264.7 cells for 24 h (with 10 ng·mL⁻¹ LPS). Data represent at least three independent experiments performed in quadruplicates. Levels of significance: ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$. The testing of **61** was done by Hannelore R cker, while the other chalcones were tested by Nafisah Al-Rifai.

Regarding the Br chalcones, both the TMC **73** and its HC analogue **97** were toxic above 1 μ M. The toxicity limit for α -Cl-TMC (**74**) was 5 μ M as shown in Table 10, but α -Cl-HC (**98**) was less toxic; *i.e.* it did not show a significant toxicity at 1-25 μ M. This reduction in toxicity is very prominent in the CF₃ case, where the α -CF₃-TMC (**77**) has a toxicity limit of 0.5 μ M (Table 10) while the HC derivative **99** was not toxic up to 50 μ M where it reduced the cell viability by 12% only. No influence on cell viability was found for the α -aryl-HC **100** at all and its TMC analogue **78** did not show toxicity in its tested range (1-25 μ M). In conclusion, it was expected that the increase in electrophilicity will lead to an increase in toxicity, but the opposite was true which again refers to the conversion of the HCs into their cyclized isomers.

NO (Griess) assay

Since the TMCs with the same X-substituents in α -CF₃-HC (**99**), α -Br-HC (**97**) and α -Cl-HC (**98**) were reported to inhibit the NO production to a large extent,¹⁰³⁻¹⁰⁴ it was assumed that the 2'-OH analogues might behave similarly. But, no activity was found in the Griess assay for α -CF₃-HC (**99**), α -Br-HC (**97**) and α -F-HC (**95**). Additionally, α -Cl-HC (**98**) inhibited the NO production with only 37% at 25 μ M. On the other hand, α -CN-HC (**95**) showed about 75% inhibition at 50 μ M and about 38% at 25 μ M, while α -CN-TMC (**69**) did not show any activity at 25 μ M. The activity of **95** may be attributed to the reversibility of the isomerization between the chalcone and its cyclized form. This is consistent with the data obtained from the LC-MS analysis of **95** in presence of cysteamine (**107**) where no flavanone was detected. The products in this case prove that the Michael addition occurred to the open form of **95**. In the case of CF₃-, Br- and Cl-substituents, the cyclization of HCs **99**, **97** and **98** cannot be reversible due to subsequent reactions of the cyclization such as the elimination of HX that was proven by the LC-MS analysis of chalcone **97** and **98** as mentioned before. The α -Me-HC (**96**) with the deactivating X substituent showed a maximum inhibition of NO formation of 42% at 50 μ M, but no inhibition at 1-10 μ M. This is just like its TMC analogue **70** which did not show any activity in this concentration range. The maximum inhibition of the α -aryl-HC **100** was 39% at 100 μ M, while at 10 μ M it showed 18% inhibition. That is lower than the inhibition of its TMC analogue **78** (36%) at the same concentration (10 μ M).

In conclusion, it was found that the enhancement of electrophilicity in α -H-HCs compared to their TMC analogues by the presence of 2'-OH group did not lead to more biological activity, but instead to a reduction or loss in their anti-inflammatory activity. This finding can be rationalized by the common conversion of the HCs into their cyclized form, *i.e.* flavanones which are not considered as Michael acceptors. In the case of the CN chalcone **95**, activity was gained due to the reversible isomerization that yields a small amount of the chalcone form which can escape from the GSH trap and subsequently reach its target. So overall, the fine-tuning regime by an α -substitution is not compatible with the 2'-OH chalcones, since the activation of the β -position will enhance the cyclization to α -X-flavanones.

RESULTS AND DISCUSSION

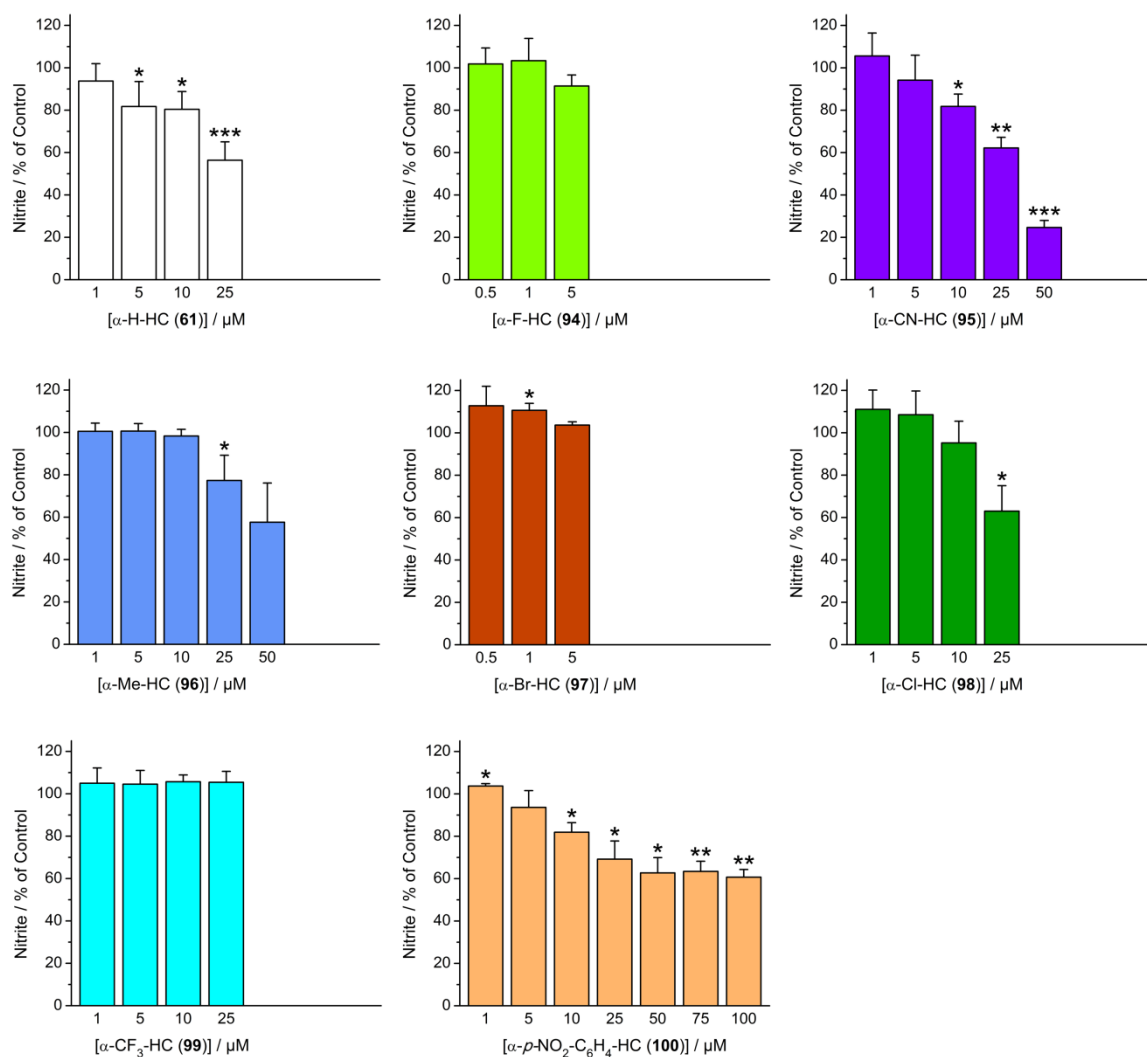


Figure 50: Results of in vitro Griess assay of α -X-HCs **61** and **94-100** with RAW264.7 cells for 24 h (with 10 ng·mL⁻¹ LPS). Data represent at least three independent experiments performed in quadruplicates. Levels of significance: ***: p < 0.001; **: p < 0.01; *: p < 0.05. The testing of **61** was done by Hannelore Rücker, while the other chalcones were tested by Nafisah Al-Rifai.

4. SUMMARY

The chalcone motif can be a privileged structure in drug design if the reactivity is modulated to reach a suitable range. Chalcones are relatively easily synthesized and they have a structural versatility that can affect their reactivity as Michael acceptors.

Since the reactivity of chalcones as Michael acceptors is essential for their biological activity, the assessment of this reactivity is of great interest. Hence, a new simple kinetic assay was developed to study the electrophilicity of chalcones upon reaction with thiols by determining the second-order rate constants. The solvent system used in the assay permits inclusion of compounds with quite diverse electrophilicity. A clear structure-activity relationship was shown within 16 α -H-chalcones.

After that, the reactivity of chalcones was fine-tuned by modifying the α -position, a strategy which is not that common in literature for this aim. A library of α -X-TMCs with different X- substituents of diverse electronic properties was prepared (Figure 51). Then the reactivity of these chalcones was assessed utilizing the developed kinetic assay.

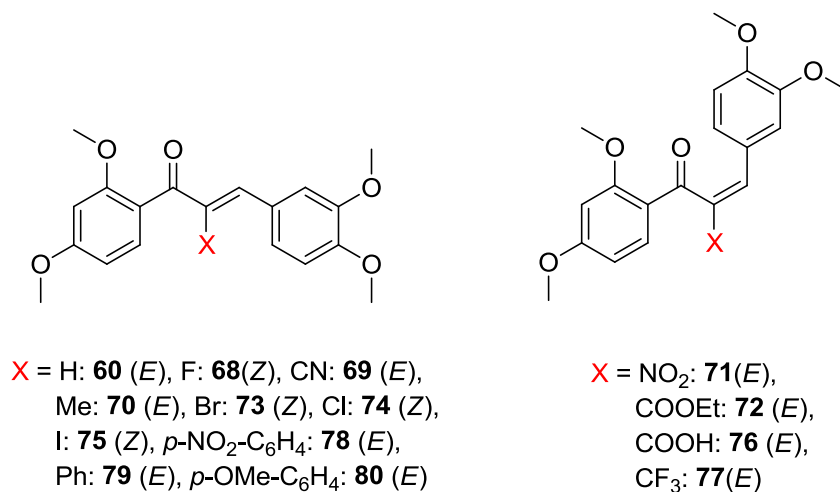
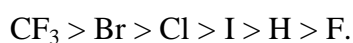


Figure 51: The synthesized α -X-TMCs.

The anti-inflammatory activity of α -X-TMCs was evaluated based on their ability to inhibit the production of NO by the proinflammatory enzyme iNOS. A strong correlation was found between Michael acceptor activity and inhibition of NO production of the α -X-TMCs for X = H, F, Cl, Br, I, CF₃. The chemical reactivity-biological activity order is:



The data prove that it is essential to find a certain reactivity window in order to retain activity. This reactivity window was identified by the kinetic assay to be in the range of $2\text{-}20\text{ M}^{-1}\text{ s}^{-1}$ for the k_2 values. So, it was proven chemically and biologically that a single simple modification on the structure of chalcones has a tremendous effect on their activity.

Finally, it was tried to enhance the activity of 2'-OH chalcones which are proven to be more active than their 2'-alkoxy analogues by α -substitution. Seven α -X-HCs (Figure 52) were synthesized and their biological activity was compared with the corresponding α -X-TMCs. The conversion of these chalcones to flavanones was proven by LC-MS analysis. It was found that this isomerization is affected by the electron-withdrawing ability of the X-substituent, *i.e.* the more this ability, the higher is the tendency of the α -X-HCs to cyclize. This cyclization made it difficult to get clean kinetics for the reaction of α -X-HCs with thiols and led to loss of activity that was supposed to be enhanced by the 2'-OH group. A gain in activity was observed only for α -CN-HC (**95**).

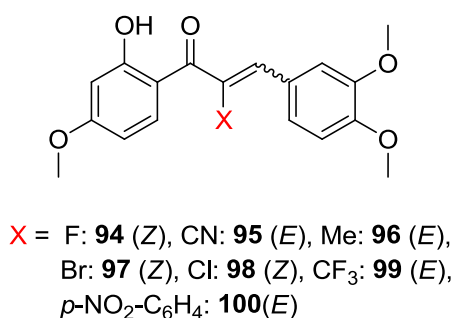


Figure 52: The synthesized α -X-HCs.

5. ZUSAMMENFASSUNG

Chalkone können als vielversprechende Strukturen für die Arzneimittelforschung betrachtet werden, wenn sich deren Reaktivitäten in einem bestimmten Rahmen befinden. Sie können relativ leicht synthetisiert werden. Aufgrund ihrer strukturellen Vielfalt ist eine Beeinflussung ihrer Michael-Akzeptor-Aktivität möglich.

Da die Reaktivität der Chalkone als Michael-Akzeptoren essentiell für ihre biologischen Aktivitäten ist, ist die Analyse dieser von großem Interesse. Deshalb wurde ein neuer, einfacher, kinetischer Assay zur Ermittlung der Elektrophilie der Chalkone durch Reaktionen mit Thiolen entwickelt. Die Analyse erfolgte durch Ermittlung der Geschwindigkeitskonstanten zweiter Ordnung (k_2). Das Lösungsmittelsystem des Assays erlaubte die Testung von Substanzen mit unterschiedlichen elektrophilen Eigenschaften. Eine deutliche Struktur-Wirkungsbeziehung wurde für 16 α -H-Chalkone gezeigt.

Im Anschluss wurden die Reaktivitäten der Chalkone durch Modifizierung der α -Position verändert. Diese Strategie ist in der Literatur mit dieser Absicht nicht üblich. Eine Substanzbibliothek von α -X-Tetramethoxychalkonen (α -X-TMCs) wurde hergestellt, wobei die X-Substituenten die elektronischen und sterischen Eigenschaften beeinflussen (Abbildung 51). Folglich wurden die Reaktivitäten dieser Chalkone mithilfe des entwickelten Kinetikassays bestimmt.

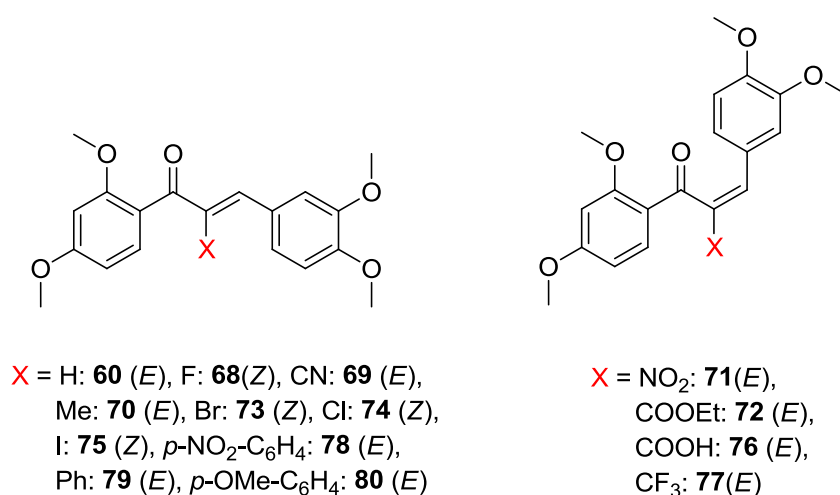


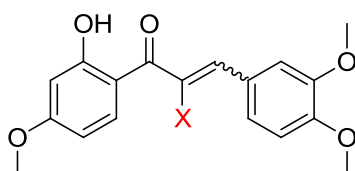
Abbildung 51: Die synthetisierten α -X-TMCs.

Die entzündungshemmende Wirkung der α -X-TMCs wurde mittels einer Hemmung des entzündungsfördernden Enzyms iNOS untersucht. Dabei wurde die NO Produktion wurde inhibiert. Eine Korrelation zwischen der Michael-Akzeptor-Aktivität und der Inhibierung der NO Produktion für die α -X-TMCs (X = H, F, Cl, Br, I, CF₃) wurde nachgewiesen. Die chemische Reaktivitäts-biologische Aktivitätsreihenfolge ist wie folgt:



Die Daten beweisen, dass die Einhaltung eines Reaktivitätsfensters essentiell zur Erhaltung der Aktivität ist. Das Reaktivitätsfenster wurde mithilfe des kinetischen Assays in einem k_2 -Wert Bereich von 2 -20 M⁻¹ s⁻¹ ermittelt. Es ist chemisch sowie biologisch bewiesen worden, dass eine einfache Modifizierung der Chalkonstruktur eine starke Änderung der Aktivität zur Folge hat.

Es wurde gezeigt, dass die Reaktivität einzelner 2'-OH Chalkone höher ist als dessen 2'-alkoxy Analoge. Durch Derivatisierung der 2'-OH Chalkone mit α -X-Substituenten wurde versucht, deren Aktivität zu verbessern. Sieben α -X-Hydroxychalkone (α -X-HCs) (Abbildung 52) wurden synthetisiert und deren biologische Aktivitäten mit den entsprechenden α -X-TMCs verglichen. Die Cyclisierung dieser Chalkone zu Flavanonen wurde durch LC-MS analysiert. Diese Isomerisierung wurde durch die elektronenziehenden Eigenschaften des X-Substituenten beeinflusst. Je elektronenziehender die Substituenten sind, desto höher ist die Tendenz zur Cyclisierung des α -X-HCs. Diese Cyclisierung erschwerte die Ermittlung der kinetischen Daten für die Reaktionen der α -X-HCs mit Thiolen. Eine Zunahme der Aktivität konnte für das α -CN-HC (**95**) erzielt werden.



X = F: **94** (Z), CN: **95** (E), Me: **96** (E),
 Br: **97** (Z), Cl: **98** (Z), CF₃: **99** (E),
 p-NO₂-C₆H₄: **100**(E)

Abbildung 52: Die synthetisierten α -X-HCs.

6. EXPERIMENTAL PART

6.1. General methods and materials

All reactions were carried out under a N₂ atmosphere in oven-heated glassware (110 °C) when dry conditions were required, and monitored by TLC on silica gel plates 60 F₂₅₄ by Merck (Darmstadt, Germany). Spots were detected under UV light (λ = 254 and 366 nm) or visualized by staining with vanillin/H₂SO₄ (6.0 g vanillin in 100 mL 95% EtOH/conc. H₂SO₄ 100:1). Column chromatography was performed on silica gel Geduran Si 60 (0.063-0.200 mm) by Merck. Preparative plates were prepared using silica gel 60 GF₂₅₄ by Merck. Melting points are determined with an automated melting point system (OptiMelt apparatus, USA) and were uncorrected. IR spectroscopy was carried on a Specac Golden Gate Diamond Single Reflection ATR System Excalibur Series FTS3000MX by Bio-Rad (Munich, Germany). NMR spectra were recorded on Bruker spectrometers (USA): Avance 300, Avance 400, and Avance III 600. ¹H NMR spectra are referenced to CDCl₃ (7.26 ppm); ¹³C NMR spectra to CDCl₃ (77.0 ppm). The following abbreviations are used to explain the multiplicities: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doublet; sept, septet; m, multiplet. Mass spectra were obtained on Agilent Technologies 6540 UHD (USA), Finnigan MAT 95 or Thermo Quest Finnigan TSQ 7000 instruments (Bremen, Germany). The samples for X-ray analysis were recrystallized from EtOAc/petroleum ether by vapor diffusion technique and the X-ray data were collected using a SuperNova, Single source at offset, Atlas diffractometer (Agilent Technologies, USA) or an Xcalibur, Ruby, Gemini Ultra diffractometer (Oxford Instruments, UK). All reagents were purchased from commercial sources and were used without further purification. Solvents were distilled before use and dried if water-free conditions were necessary. The used petroleum ether is the one with a boiling range of 40-60 °C. Ethylene glycol was used in spectrophotometric grade from Sigma-Aldrich (Taufkirchen, Germany).

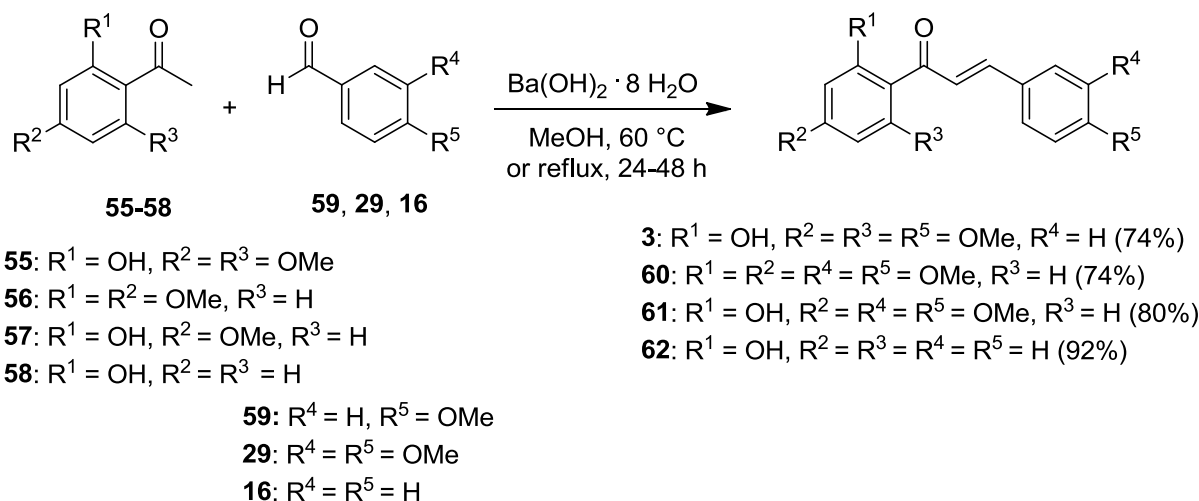
Chalcone (**1**) was from Merck (Darmstadt, Germany) and xanthohumol (**5**) from Carl Roth (Karlsruhe, Germany). The other α -H-chalcones which were tested in the kinetic thiol assay and were not synthesized during this PhD work were prepared by my colleagues in our group. The cysteamine (**107**) that was used in the thiol assay is a hydrochloride salt (*i.e.* cysteamine hydrochloride).

6.2. Synthetic procedures*

6.2.1. General procedure for the synthesis of chalcones α -H-chalcones **3**, **60-62**,⁶⁶ α -F-TMC (**68**) and α -Me-TMC (**70**)

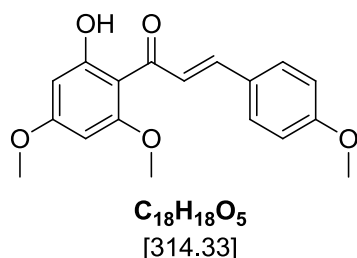
Benzaldehydes (1.0 eq.) and the corresponding dimethoxyacetophenones (1.0 eq.) were dissolved in MeOH (0.2 M). Ba(OH)₂·8 H₂O (1.0 eq.) was added and the reaction mixture was stirred at 60 °C or under reflux. The reaction mixture was subsequently concentrated *in vacuo*. Then H₂O was added, the resulting solution was neutralized with 1 M aq. HCl, and extracted with EtOAc. The combined organic layers were dried over MgSO₄ and evaporated under reduced pressure. The product was purified by column chromatography, on preparative TLC plates, or by recrystallization to afford the corresponding chalcones.

α -H-chalcones **3**, **60-62**



(*E*)-1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one

(Flavokawain A) (3). Flavokawain A (**3**) was prepared according to the general procedure (**5.2.1**) starting from 1-(2-hydroxy-4,6-dimethoxyphenyl)-ethanone (**55**) (300 mg, 1.53 mmol, 1.0 eq.) and 4-methoxybenzaldehyde (**59**) (208 mg, 1.53 mmol, 1.0 eq.). The reaction mixture was stirred at 60 °C for 24 h. Purification by recrystallization from MeOH afforded chalcone **3** (354 mg, 1.13 mmol, 74%) as yellow crystals.



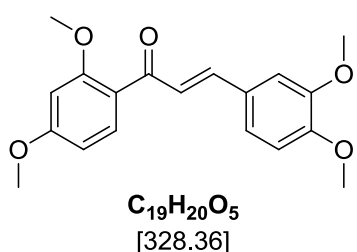
* Structures of the synthesized compounds in this study with numbers are listed in Appendix 6.1 for easy referral. The X-Ray data are given in Appendix 6.2 and the NMR spectra in Appendix 6.3.

¹H NMR (400 MHz, CDCl₃): δ = 14.41 (s, 1 H; 2'-OH), 7.82 (d, J = 15.6 Hz, 1 H; β -CH), 7.77 (d, J = 15.7 Hz, 1 H, α -CH), 7.63-7.52 (m, 2 H), 7.04-6.82 (m, 2 H), 6.11 (d, J = 2.4 Hz, 1 H), 5.96 (d, J = 2.4 Hz, 1 H), 3.92 (s, 3 H; OCH₃), 3.85 (s, 3 H, OCH₃), 3.84 (s, 3 H, OCH₃) ppm.

¹³C NMR (75 MHz, CDCl₃): δ = 192.6 (C=O), 168.4, 166.0, 162.5, 161.4, 142.5 (β -CH), 130.1 (2 CH), 128.3, 125.1 (α -CH), 114.4 (2 CH), 106.4, 93.8 (CH), 91.2 (CH), 55.9 (OCH₃), 55.6 (OCH₃), 55.4 (OCH₃) ppm.

The NMR spectral data are in accordance with the literature.¹⁰⁶

(*E*)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (α -H-TMC, **60).**



Chalcone **60** was prepared according to the general procedure (**5.2.1**) starting from 1-(2,4-dimethoxyphenyl)ethanone (**56**) (3.60 g, 20.0 mmol, 1.0 eq.) and 3,4-dimethoxybenzaldehyde (**29**) (3.30 g, 20.0 mmol, 1.0 eq.). The reaction mixture was stirred at 60 °C for 24 h. Purification by column chromatography

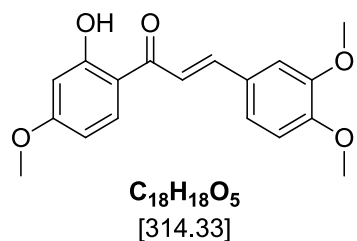
(SiO₂, petroleum ether/EtOAc, 3:1) afforded α -H-TMC (**60**) (4.84 g, 14.7 mmol, 74%) as a yellow solid; m.p. 81 °C (recrystallized from EtOAc/petroleum ether); lit. m.p. 89 °C.¹⁰⁷

¹H NMR (300 MHz, CDCl₃): δ = 7.73 (d, J = 8.6 Hz, 1 H), 7.62 (d, J = 15.7 Hz, 1 H; β -H), 7.35 (d, J = 15.7 Hz, 1 H; α -H), 7.19 (dd, J = 8.3, 1.9 Hz, 1 H), 7.12 (d, J = 1.9, 1 H), 6.88 (d, J = 8.3 Hz, 1 H), 6.57 (dd, J = 8.6, 2.3 Hz, 1 H), 6.50 (d, J = 2.2 Hz, 1 H), 3.92 (s, 3 H; OCH₃), 3.92 (s, 3 H; OCH₃), 3.90 (s, 3 H; OCH₃), 3.87 (s, 3 H; OCH₃) ppm.

¹³C NMR (75 MHz, CDCl₃): δ = 190.8 (C=O), 164.0, 160.2, 151.0, 149.1, 142.5 (β -CH), 132.7 (CH), 128.4, 125.3 (α -CH), 122.6 (CH), 122.5, 111.1 (CH), 110.2 (CH), 105.1 (CH), 98.8 (CH), 56.0 (OCH₃), 55.9 (OCH₃), 55.8 (OCH₃), 55.6 (OCH₃) ppm.

The ¹H NMR spectral data are in accordance with the literature.³⁶

(*E*)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxy-phenyl)prop-2-en-1-one



(α -H-HC, **61**). The 2'-hydroxychalcone **61** was prepared according to the general procedure (**5.2.1**) starting from 1-(2-hydroxy-4-methoxyphenyl)ethanone (**57**) (2.50 g, 15.1 mmol, 1.0 eq.) and 3,4-dimethoxybenzaldehyde (**29**) (2.50 g, 15.1 mmol, 1.0 eq.). The reaction mixture was heated to reflux for

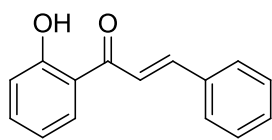
48 h. Purification by recrystallization from acetone afforded α -H-HC (**61**) (3.78 g, 12.0 mmol, 80%) as yellow crystals; m.p. 160 °C; lit. m.p. 170 °C (recrystallized from MeOH).¹⁰⁸

¹H NMR (300 MHz, CDCl₃): δ = 13.56 (s, 1 H; 2'-OH), 7.87-7.81 (m, 2 H), 7.43 (d, J = 15.4 Hz, 1 H), 7.24 (dd, J = 8.4, 1.9, 1 H), 7.15 (d, J = 1.9 Hz, 1 H), 6.90 (d, J = 8.3 Hz, 1 H), 6.50-6.47 (m, 2 H), 3.96 (s, 3 H; OCH₃), 3.93 (s, 3 H; OCH₃), 3.85 (s, 3 H; OCH₃) ppm.

¹³C NMR (75 MHz, CDCl₃): δ = 191.8 (C=O), 166.7, 166.1, 151.6, 149.3, 144.6 (β -CH), 131.1 (CH), 127.8, 55.6 (OCH₃), 123.4 (CH), 118.0 (α -CH), 114.1, 111.1 (CH), 110.2 (CH), 107.7 (CH), 101.0 (CH), 56.0 (OCH₃), 56.0 (OCH₃) ppm.

The ¹H NMR spectral data are in accordance with the literature.¹⁰⁸

(*E*)-1-(2-Hydroxyphenyl)-3-phenylprop-2-en-1-one (62). Chalcone **62** was prepared



C₁₅H₁₂O₂
[224.25]

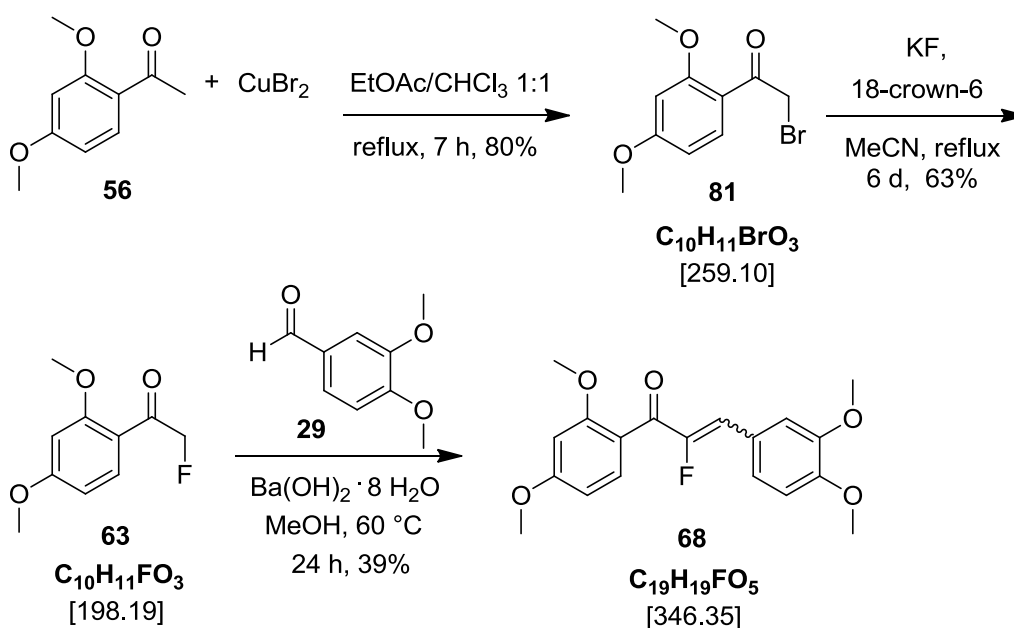
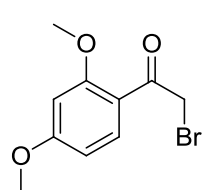
according to the general procedure (5.2.1) starting from 1-(2-hydroxyphenyl)ethanone (**58**) (640 mg, 4.70 mmol, 1.0 eq.) and benzaldehyde (**16**) (500 mg, 4.70 mmol, 1.0 eq.). The reaction mixture was stirred at 60 °C for 24 h. Purification by recrystallization from MeOH afforded the yellow 2'-hydroxychalcone (**62**) (967 mg,

4.31 mmol, 92%).

¹H NMR (300 MHz, CDCl₃): δ = 12.82 (s, 1 H; 2'-OH), 7.94 (d, J = 15.5 Hz, 1 H; β -CH), 7.94 (dd, J = 8.1, 1.6 Hz, 1 H), 7.67 (d, J = 15.4 Hz, 1 H; α -CH), 7.69-7.67 (m, 2 H), 7.51 (ddd, J = 8.6, 7.2, 1.6 Hz, 1 H), 7.48-7.42 (m, 3 H), 7.04 (dd, J = 8.4, 1.1 Hz, 1 H), 6.96 (ddd, J = 8.2, 7.2, 1.1 Hz, 1 H) ppm.

¹³C NMR (75 MHz, CDCl₃): δ = 193.7 (C=O), 163.6, 145.5 (β -CH), 136.4 (CH), 134.6, 130.9 (CH), 129.6 (α -CH), 129.0 (2 CH), 128.7 (2 CH), 120.1 (CH), 120.0, 118.8 (CH), 118.6 (CH) ppm.

The ¹H NMR spectral data are in accordance with the literature.¹⁰⁹

(Z/E)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-fluoroprop-2-en-1-one (α -F-TMC, 68)**2-Bromo-1-(2,4-Dimethoxyphenyl)ethanone (81).** 1-(2,4-Dimethoxyphenyl)ethanone (**56**)

(1.30 g, 7.21 mmol, 1.0 eq.) was dissolved in 1.5 L of a $\text{CHCl}_3/\text{EtOAc}$ mixture (1:1). The mixture was heated to reflux. CuBr_2 (3.30 g, 14.8 mmol, 2.05 eq.) was added in three portions over 3 h. The reflux was stopped after 7 h of the first addition. The mixture was cooled to room temperature then

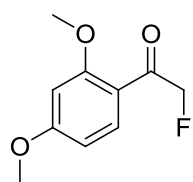
filtered over silica gel and washed with EtOAc . The solvent was evaporated *in vacuo* and the residue was subjected to column chromatography (SiO_2 , petroleum ether/ CH_2Cl_2 , 1:1) to yield 2-bromoacetophenone **81** as a white solid (1.50 g, 5.79 mmol, 80%).

$^1\text{H NMR}$ (300 MHz, CDCl_3): δ = 7.92 (d, J = 8.8 Hz, 1 H), 6.57 (dd, J = 8.8, 2.3 Hz, 1 H), 6.47 (d, J = 2.3 Hz, 1 H), 4.58 (s, 2 H; CH_2Br), 3.93 (s, 3 H; OCH_3), 3.87 (s, 3 H; OCH_3) ppm.

$^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ = 190.2 (C=O), 165.4, 160.9, 133.8 (CH), 117.9, 105.9 (CH), 98.3 (CH), 55.7 (OCH_3), 55.7 (OCH_3), 38.0 (CH_2Br) ppm.

$^1\text{H NMR}$ spectral data is in accordance with the literature data.¹¹⁰

1-(2,4-Dimethoxyphenyl)-2-fluoroethanone (63).¹³ To freshly dried KF (3.36 g, 57.9 mmol, 20.0 eq.), 2-bromoacetophenone **81** (750 mg, 2.89 mmol, 1.0 eq.) and 18-crown-6 (153 mg, 0.579 mmol, 0.2 eq.) in dry MeCN (200 mL) were added under a N₂ atmosphere. The mixture was refluxed for 6 days. The solvent was then removed *in vacuo* and the residue partitioned between CH₂Cl₂ and H₂O. The organic fraction was dried and evaporated to gain a yellow oil. Purification by column chromatography (SiO₂, petroleum ether/EtOAc, 4:1) yielded 2-fluoroacetophenone **63** as a white solid (361 mg, 1.82 mmol, 63%); m.p. 142 °C.



¹H NMR (400 MHz, CDCl₃): δ = 8.05 (d, J = 8.8 Hz, 1 H), 6.60 (dd, J = 8.8, 2.2 Hz, 1 H), 6.45 (d, J = 2.2 Hz, 1 H), 5.38 (d, J = 48.3 Hz, 2 H; CH₂F), 3.91 (s, 3 H; OCH₃), 3.87 (s, 3 H; OCH₃) ppm.

¹³C NMR (75 MHz, CDCl₃): δ = 192.1 (d, J = 14.2 Hz, CH₂F-C=O), 165.6, 161.5, 133.0 (d, J = 2.7 Hz, CH₂F-C(O)-C-CH), 117.1, 106.1 (CH), 98.0 (CH), 86.2 (d, J = 178.0 Hz, CH₂F), 55.7 (OCH₃), 55.6 (OCH₃) ppm.

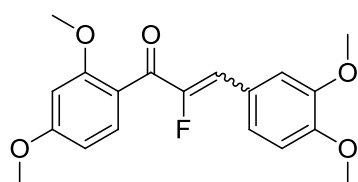
¹⁹F-NMR (376 MHz, CDCl₃): δ = -224.3 (t, J = 48 Hz) ppm.

IR (neat): 1665, 1602, 1225, 1211, 1018, 969, 834 cm⁻¹.

MS (EI): m/z (%): 198 [M⁺] (17), 165 (100).

HRMS (EI): calcd for C₁₀H₁₁FO₃: 198.0692 [M⁺]; found 198.0694.

(Z/E)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-fluoroprop-2-en-1-one (α-F-TMC, 68).



The α-fluoro chalcone **68** was prepared according to the general procedure (5.2.1) starting from 2-fluoroacetophenone **63** (99.0 mg, 0.500 mmol, 1.0 eq.) and 3,4-dimethoxybenzaldehyde (**29**) (83.0 mg, 0.500 mmol, 1.0 eq.), which were dissolved in dry MeOH under a N₂ atmosphere. The suspension was stirred at 60 °C for 24 h. Column chromatography (SiO₂, petroleum ether/EtOAc, 7:3) afforded α-F-TMC (**68**) as a yellow solid (67.0 mg, 0.194 mmol, 39%) as a Z/E mixture of 92:8; m.p. 112 °C.

Z-68: **¹H NMR** (600 MHz, CDCl₃): δ = 7.40 (d, J = 8.4 Hz, 1 H), 7.27 (s, 1 H), 7.20 (dd, J = 8.4, 1.9 Hz, 1 H), 6.87 (d, J = 8.4 Hz, 1 H), 6.64 (d, J = 35.6 Hz, 1 H; β-H), 6.54 (dd, J = 8.5,

2.2 Hz, 1 H), 6.51 (d, $J = 2.2$ Hz, 1 H), 3.91 (s, 3 H; OCH₃), 3.89 (s, 3 H; OCH₃), 3.86 (s, 3 H; OCH₃), 3.82 (s, 3 H; OCH₃) ppm.

Z-68: ¹³C NMR (101 MHz, CDCl₃): $\delta = 187.7$ (d, $J = 30.0$ Hz, C=O), 163.6, 159.6, 154.1 (d, $J = 267.7$ Hz, α -C), 150.5 (d, $J = 3.4$ Hz), 148.9, 131.5 (CH), 124.61 (d, $J = 4.8$ Hz), 124.60 (d, $J = 7.3$ Hz, CH), 120.0, 119.2 (d, $J = 6.4$ Hz, β -CH), 112.8 (d, $J = 10.0$ Hz, CH), 111.0 (CH), 104.7 (CH), 98.8 (CH), 55.9 (OCH₃), 55.8 (OCH₃), 55.8 (OCH₃), 55.5 (OCH₃) ppm.

Z-68: ¹⁹F NMR (376 MHz, CDCl₃): $\delta = -124.2$ (d, $J = 35.7$ Hz) ppm.

E-68: ¹H NMR (600 MHz, CDCl₃): $\delta = 7.58$ (d, $J = 8.6$ Hz, 1 H), 7.17 (d, $J = 2.0$ Hz, 1 H), 6.95 (dd, $J = 8.3, 1.9$ Hz, 1 H), 6.74 (d, $J = 8.3$ Hz, 1 H), 6.63 (d, $J = 23.7$ Hz, 1 H; β -H), 6.48 (dd, $J = 8.6, 2.3$ Hz, 1 H), 6.38 (d, $J = 2.2$ Hz, 1 H), 3.84 (s, 3 H; OCH₃), 3.82 (s, 3 H; OCH₃), 3.81 (s, 3 H; OCH₃), 3.81 (s, 3 H; OCH₃) ppm.

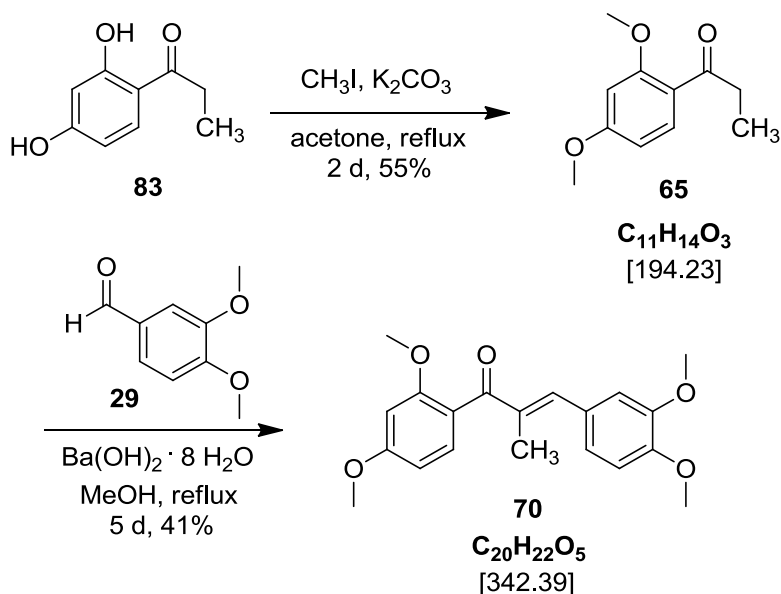
E-68: ¹⁹F NMR (376 MHz, CDCl₃): $\delta = -111.3$ (d, $J = 23.8$ Hz) ppm.

IR (Z/E-68, neat): 1652, 1632, 1604, 1519, 1333, 1260, 1211, 1025 cm⁻¹.

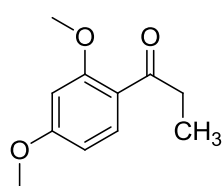
MS (EI): m/z (%): 346 [M⁺] (99), 165 (100).

HRMS (EI): calcd for C₁₉H₁₉FO₅: 346.1217 [M⁺]; found 346.1213.

(E)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-methylprop-2-en-1-one (α -Me-TMC, **70**)



1-(2,4-Dimethoxyphenyl)propan-1-one (65).⁷¹ Anhydrous K₂CO₃ (1.80 g, 13.2 mmol, 2.2



eq.) was added to a stirred solution of 1-(2,4-dihydroxyphenyl)ethanone (**83**) (1.00 g, 6.00 mmol, 1.0 eq.) in dry acetone (15 mL). After a few minutes, CH₃I (7.5 mL, 120 mmol, 20.0 eq.) was added to the suspension.

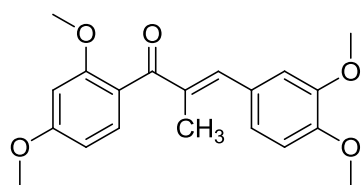
The reaction mixture was heated to reflux for 2 days, cooled, filtered and the remaining solid was washed with hot acetone. After evaporating the solvent *in vacuo*, the residue was dissolved in CH₂Cl₂, washed with H₂O, dried over MgSO₄ and the solvent evaporated. The crude product was subjected to column chromatography (SiO₂, petroleum ether/EtOAc, 10:1) to give 2-methylacetophenone **65** (646 mg, 3.33 mmol, 55%) as a white solid.

¹H NMR (300 MHz, CDCl₃): δ = 7.79 (d, J = 8.7 Hz, 1 H), 6.50 (dd, J = 8.7, 2.3 Hz, 1 H), 6.43 (d, J = 2.3 Hz, 1 H), 3.86 (s, 3 H; OCH₃), 3.82 (s, 3 H; OCH₃), 2.94 (q, J = 7.3 Hz, 2 H; CH₂), 1.13 (t, J = 7.3 Hz, 3 H; CH₃) ppm.

¹³C NMR (75 MHz, CDCl₃): δ = 201.0 (C=O), 164.1, 160.6, 132.5 (CH), 121.0, 104.9 (CH), 98.2 (CH), 55.4 (OCH₃), 55.3 (OCH₃), 36.7 (CH₂), 8.5 (CH₃) ppm.

The ¹H NMR spectral data is in accordance with the literature.¹¹¹

(E)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-methylprop-2-en-1-one (α-Me-TMC, 70).



The α-methylchalcone **70** was prepared according to the general procedure (5.2.1) starting from 2-methylacetophenone **65** (350 mg, 1.80 mmol, 1.0 eq.) and 3,4-dimethoxybenzaldehyde (**29**) (300 mg, 1.80 mmol, 1.0 eq.). The

reaction mixture was stirred at reflux for 5 days. Purification on preparative TLC plates (SiO₂, petroleum ether/EtOAc, 7:3) afforded a sticky solid material, which was precipitated from petroleum ether to give α-Me-TMC (**70**) (250 mg, 0.730 mmol, 41%) as a pale yellow solid; m.p. 71 °C; the configuration of the double bond was assigned according to the literature.⁷⁸

¹H NMR (400 MHz, CDCl₃): δ = 7.28 (d, J = 8.2 Hz, 1 H), 7.11 (br s, 1 H; β-CH), 7.03 (dd, J = 8.4, 1.9 Hz, 1 H), 6.92 (d, J = 1.9 Hz, 1 H), 6.88 (d, J = 8.4 Hz, 1 H), 6.54-6.51 (m, 2 H), 3.91 (s, 3 H; OCH₃), 3.88 (s, 3 H; OCH₃), 3.86 (s, 3 H; OCH₃), 3.79 (s, 3 H; OCH₃), 2.24 (d, J = 1.2 Hz, 3 H; CH₃) ppm.

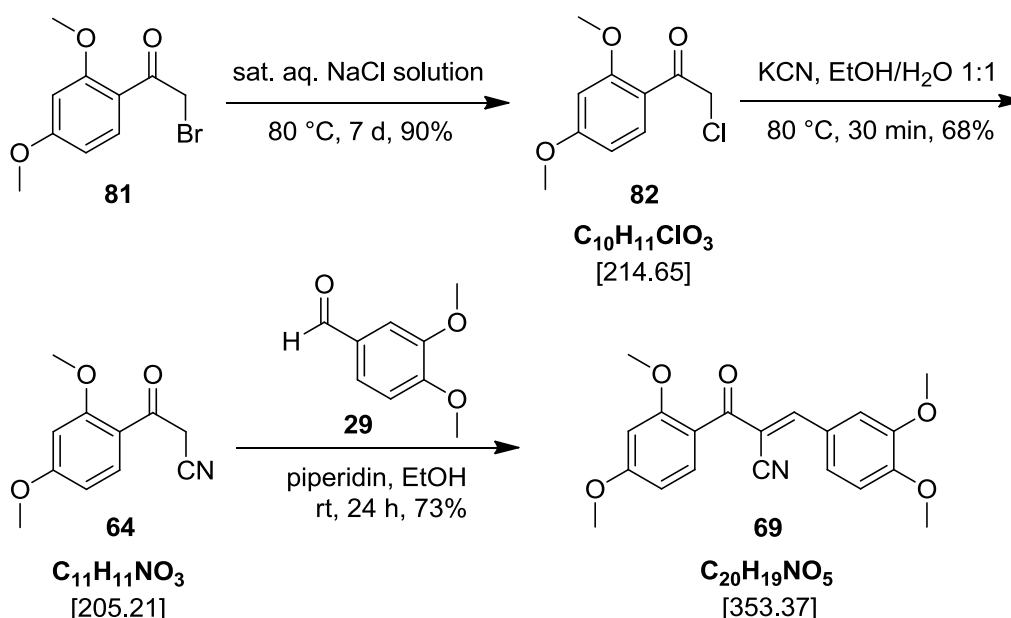
^{13}C NMR (101 MHz, CDCl_3): δ = 198.5 (C=O), 162.4, 158.8, 149.4, 148.6, 142.3 (β -CH), 136.7, 130.9 (CH), 129.1, 123.4 (CH), 122.4, 113.0 (CH), 110.9 (CH), 104.3 (CH), 98.9 (CH), 55.9 (OCH_3), 55.9 (OCH_3), 55.7 (OCH_3), 55.5 (OCH_3), 13.6 (CH_3) ppm.

IR (neat): 1616, 1595, 1514, 1262, 1212, 1021, 1010, 819 cm^{-1} .

MS (ESI^+): m/z (%): 343 $[\text{M}+\text{H}]^+$ (100).

HRMS (ES-MS): calcd for $\text{C}_{20}\text{H}_{23}\text{O}_5$: 343.1540 $[\text{M}+\text{H}]^+$; found 343.1545.

6.2.2. Synthesis of (*E*)-2-cyano-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (α -CN-TMC, **69**)



2-Chloro-1-(2,4-dimethoxyphenyl)ethanone (82**)**. A saturated aqueous solution of NaCl (70 mL) was added to 2-bromoacetophenone **81** (710 mg, 2.74 mmol) in a round-bottom flask. The resulting mixture was heated at 80 °C for 7 days. After cooling to room temperature the mixture was extracted with EtOAc (5 \times 50 mL), the organic extract dried over MgSO_4 and the solvent was evaporated. The product was then purified on preparative TLC plates (petroleum ether/EtOAc, 4:1) to afford 2-chloroacetophenone **82** (530 mg, 2.47 mmol, 90%) as a white solid.

^1H NMR (300 MHz, CDCl_3): δ = 7.94 (d, J = 8.8, 1 H), 6.56 (dd, J = 8.8, 2.3 Hz, 1 H), 6.45 (d, J = 2.3 Hz, 1 H), 4.74 (s, 2 H; CH_2Cl), 3.91 (s, 3 H; OCH_3), 3.86 (s, 3 H; OCH_3) ppm.

^{13}C NMR (75 MHz, CDCl_3): δ = 190.0 (C=O), 165.4, 160.9, 133.5 (CH), 117.9, 105.9 (CH), 98.1 (CH), 55.6 (2 OCH_3), 51.3 (CH_2Cl) ppm.

The ^1H NMR spectral data are in accordance with the literature data.¹¹²

3-(2,4-Dimethoxyphenyl)-3-oxopropanenitrile (64**)**.⁷⁰ KCN (455 mg, 6.99 mmol, 5.0 eq.) in H_2O (1.4 mL) was added to a solution of 2-chloroacetophenone **82** (300 mg, 1.40 mmol, 1.0 eq.) in EtOH (1.4 mL). The resulting mixture was gradually heated in a water bath up to 80 °C until the solution was brown (~ 30 min). After cooling to room temperature, the reaction mixture was diluted with H_2O (30 mL), acidified with 1 M aq. HCl (pH 4-5), and cooled in an ice-bath. The formed precipitate was filtered and crystallized from EtOH to get **64** (130 mg) as light brown crystals. The filtrate was extracted with CH_2Cl_2 (5 \times 30 mL), the organic extract dried over MgSO_4 and the solvent evaporated under reduced pressure. The product was purified on preparative TLC plates (petroleum ether/EtOAc, 7:3) to afford another crop of **64** (66 mg). The total yield of 2-cyanoacetophenone **64** was 196 mg (0.955 mmol, 68%).

^1H NMR (300 MHz, CDCl_3): δ = 7.94 (d, J = 8.9 Hz, 1 H), 6.59 (dd, J = 8.8, 2.3 Hz, 1H), 6.47 (d, J = 2.3 Hz, 1 H), 4.03 (s, 2 H; CH_2CN), 3.95 (s, 3 H; OCH_3), 3.88 (s, 3 H; OCH_3) ppm.

^{13}C NMR (101 MHz, CDCl_3): δ = 185.9 (C=O), 166.0, 161.2, 133.6 (CH), 117.7, 114.9 (CN), 106.2 (CH), 98.2 (CH), 55.7 (2 OCH_3), 33.9 (CH_2CN) ppm.

The ^1H NMR spectral data are in accordance with the literature data.¹¹²

(*E*)-2-Cyano-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (α -CN-TMC, **69)**.¹³

The 2-cyanoacetophenone **64** (100 mg, 0.478 mmol, 1.0 eq.) and 3,4-dimethoxybenzaldehyde (**29**) (81.0 mg, 0.478 mmol, 1.0 eq.) were warmed in EtOH (50 mL) using the heating gun until they dissolved. The solution was then cooled to room temperature and piperidine (100 μL , 1.01 mmol, 2.1 eq.) was added. After 24 h of stirring at this temperature, EtOH was removed under vacuum and the residue partitioned between CH_2Cl_2 and H_2O . The CH_2Cl_2 fraction was dried over MgSO_4 and evaporated. The product was purified on preparative TLC plates (petroleum ether/EtOAc, 7:3) to afford α -CN-TMC (**69**) (126 mg, 0.357 mmol, 73%) as a yellow solid that was recrystallized from a

EtOAc/petroleum ether mixture affording yellow crystals of the pure *E* isomer (100 mg, 0.283 mmol, 58%); m.p. 139 °C.

¹H NMR (300 MHz, CDCl₃): δ = 7.99 (s, 1 H; β -H), 7.82 (d, J = 2.1 Hz, 1 H), 7.55-7.47 (m, 2 H), 6.95 (d, J = 8.5 Hz, 1 H), 6.58 (dd, J = 8.5, 2.2 Hz, 1 H), 6.51 (d, J = 2.2 Hz, 1 H), 3.97 (s, 3 H; OCH₃), 3.96 (s, 3 H; OCH₃), 3.88 (s, 3 H; OCH₃), 3.88 (s, 3 H; OCH₃) ppm.

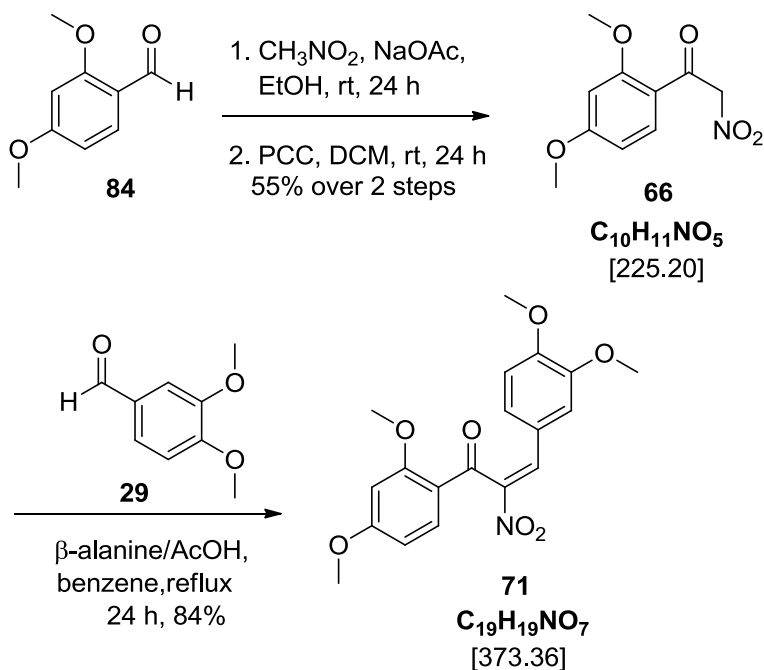
¹³C NMR (75 MHz, CDCl₃): δ = 188.1 (C=O), 164.5, 159.8, 153.3, 152.6 (β -CH), 149.2, 132.2 (CH), 127.6 (CH), 125.3, 120.2, 118.1, 111.9 (CH), 110.9 (CH), 109.6, 105.5 (CH), 98.5 (CH), 56.1 (OCH₃), 56.0 (OCH₃), 55.7 (OCH₃), 55.6 (OCH₃) ppm.

IR (neat): 2932, 2839, 2205, 1658, 1596, 1255, 1213, 1114, 1020, 834 cm⁻¹.

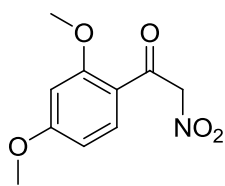
MS (EI): m/z (%): 353 [M^{+}] (31), 165 (100).

HRMS (EI): calcd for C₂₀H₁₉NO₅: 353.1263 [M^{+}]; found 353.1258.

6.2.3. Synthesis of (*E*)-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-nitroprop-2-en-1-one (α -NO₂-TMC, 71)



1-(2,4-Dimethoxyphenyl)-2-nitroethanone (66).⁷⁵ 2,4-Dimethoxybenzaldehyde (**84**) (300



mg, 1.81 mmol, 1.0 eq.) was added to a solution of CH_3NO_2 (1.40 mL, 25.3 mmol, 14 eq.) in abs. EtOH (8 mL). NaOAc (2.08 g, 25.3 mmol, 14 eq.) was added to the reaction mixture and stirred at room temperature for

24 h. After that, EtOH and excess CH_3NO_2 were evaporated using a N_2

stream. H_2O (20 mL) was added to the residue and the mixture was extracted with EtOAc (3×10 mL), the organic extract dried over MgSO_4 and the solvent evaporated under reduced pressure to afford 335 mg (1.47 mmol) of the corresponding nitroalcohol,¹¹³ that was directly used in the next step. It was dissolved in dry CH_2Cl_2 (10 mL) and cooled to 0°C , then pyridinium chlorochromate (PCC) (759 mg, 3.52 mmol, 2.4 eq.) was added and the reaction was allowed to warm up to room temperature overnight. The solvent mixture was filtered and the solvent evaporated under reduced pressure. The product was purified on preparative TLC plates (petroleum ether/EtOAc, 1:1) to afford 2-nitroacetophenone **66** (224 mg, 0.995 mmol, 55% over 2 steps) as a beige solid; m.p. 130°C .

^1H NMR (400 MHz, CDCl_3): δ = 8.02 (d, J = 8.9 Hz, 1 H), 6.61 (dd, J = 8.9, 2.2 Hz, 1 H), 6.46 (d, J = 2.2 Hz, 1 H), 5.73 (s, 2 H; CH_2NO_2), 3.92 (s, 3 H; OCH_3), 3.89 (s, 3 H; OCH_3) ppm.

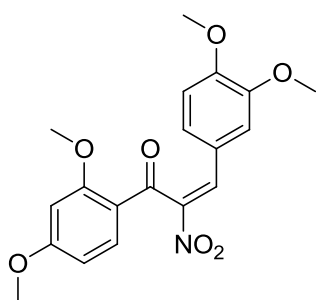
^{13}C NMR (101 MHz, CDCl_3): δ = 184.1 (C=O), 166.5, 161.5, 133.7 (CH), 116.8, 106.6 (CH), 98.1 (CH), 85.2 (CH_2NO_2), 55.8 (OCH_3), 55.7 (OCH_3) ppm.

IR (neat): 3031, 2940, 1651, 1595, 1553, 1453, 1392, 1253, 1212, 1021, 828 cm^{-1} .

MS (EI): m/z (%): 225 [M^{+}] (32), 165 (100).

HRMS (EI): calcd. for $\text{C}_{10}\text{H}_{11}\text{NO}_5$: 225.0637 [M^{+}]; found 225.0635.

(E)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-nitroprop-2-en-1-one (α -NO₂-TMC, **71).**⁵⁹ A mixture of 2-nitroacetophenone **66** (589 mg, 2.62



mmol, 1.0 eq.), 3,4-dimethoxybenzaldehyde (**29**) (696 mg, 4.19 mmol, 1.6 eq.), β -alanine (19.6 mg, 0.220 mmol, 0.084 eq.) and glacial AcOH (420 μL , 7.34 mmol, 2.8 eq.) in benzene (15 mL) was heated to reflux overnight by using a Dean Stark apparatus.

The reaction mixture was washed with H_2O (3×10 mL) then the aqueous layer was extracted with CH_2Cl_2 (3×10 mL), the organic extracts were combined and dried over MgSO_4 . The solvent was evaporated *in vacuo* and the resulting residue was

subjected to column chromatography (SiO₂, petroleum ether/EtOAc, 7:3) to afford α -NO₂-TMC (**71**) (820 mg, 2.20 mmol, 84%) as a yellow solid; m.p. 44 °C. The stereochemistry of chalcone **71** was assigned as the *E* isomer based on literature.⁷⁹

¹H NMR (400 MHz, CDCl₃): δ = 8.15 (d, *J* = 8.9 Hz, 1 H), 7.94 (s, 1 H; β -H), 7.08 (dd, *J* = 8.5, 2.0 Hz, 1 H), 6.91 (d, *J* = 2.1 Hz, 1 H), 6.81 (d, *J* = 8.4 Hz, 1 H), 6.63 (dd, *J* = 8.9, 2.3 Hz, 1 H), 6.41 (d, *J* = 2.3 Hz, 1 H), 3.88 (s, 6 H; 2 OCH₃), 3.76 (s, 3 H; OCH₃), 3.68 (s, 3 H; OCH₃) ppm.

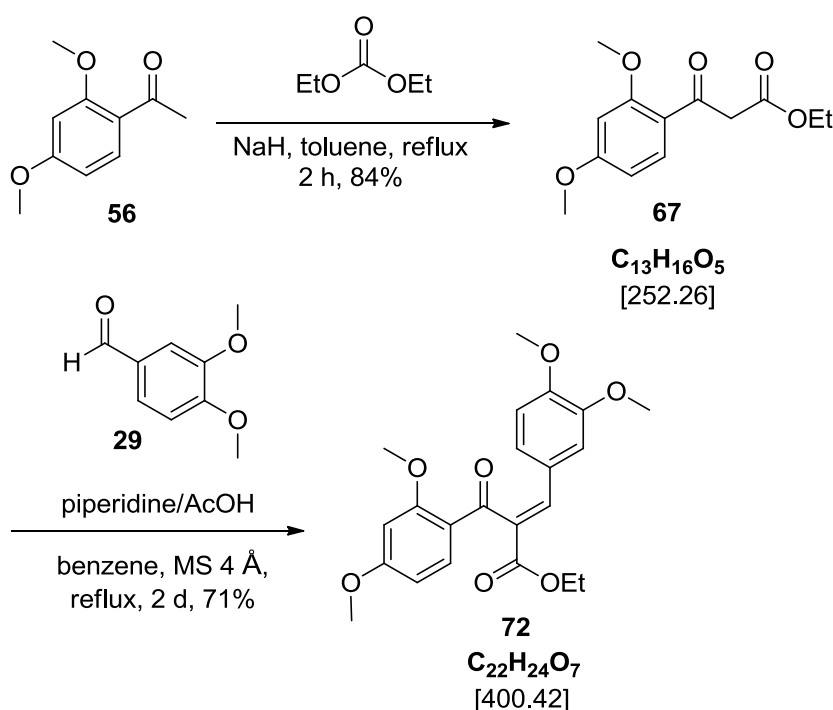
¹³C NMR (101 MHz, CDCl₃): δ = 184.3 (C=O), 166.6, 162.2, 152.1, 149.0, 147.5, 133.5 (CH), 133.2 (β -CH), 126.3 (CH), 122.6, 118.6, 112.3 (CH), 111.1 (CH), 106.8 (CH), 98.3 (CH), 56.0 (OCH₃), 55.9 (OCH₃), 55.7 (OCH₃), 55.6 (OCH₃) ppm.

IR (neat): 2939, 2839, 1660, 1590, 1508, 1313, 1249, 1018 cm⁻¹.

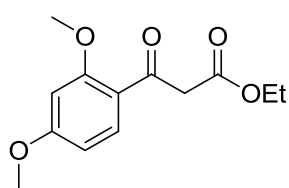
MS (ESI⁺): *m/z* (%): 374 [M+H]⁺ (100).

HRMS (ES-MS): calcd for C₁₉H₂₀NO₇: 374.1234 [M+H]⁺; found 374.1235.

6.2.4. Synthesis of (*E*)-2-ethoxycarbonyl-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)propen-1-one (α -COOEt-TMC, **72**)



Ethyl 3-(2,4-dimethoxyphenyl)-3-oxopropanoate (67**).**⁷⁶ NaH (1.24 g (60% in paraffin oil),



31.0 mmol, 2.8 eq.) was added to a solution of diethyl carbonate (2.60 g, 22.0 mmol, 2.0 eq.) in toluene (11.0 mL). The mixture was heated to reflux then a solution of 1-(2,4-dimethoxyphenyl)ethanone (**56**) (2.00 g, 11.0 mmol, 1.0 eq.) in toluene (5.5 mL) was added

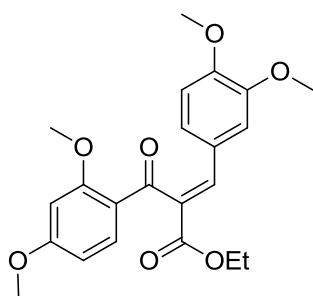
dropwise over about 1.5 h. After the addition, the reflux was continued until the evolution of hydrogen ceased (30 min). Subsequently, the reaction was cooled to room temperature and glacial AcOH (3.3 mL) was added dropwise leading to a precipitate that was dissolved upon addition of cold H₂O (30 mL). The toluene layer was separated, and the aqueous layer was extracted with EtOAc (3 × 35 mL). The combined organic solution was washed with H₂O (35 mL) and brine (35 mL), and finally dried over MgSO₄. After evaporation of the solvent *in vacuo*, the mixture was subjected to column chromatography (SiO₂, petroleum ether/EtOAc, 5:1) to give the desired β -keto ester **67** (2.32 g, 9.20 mmol, 84%) as a pale yellow oil.

¹H NMR (300 MHz, CDCl₃): δ = 7.93 (d, J = 8.8 Hz, 1 H), 6.54 (dd, J = 8.8, 2.3 Hz, 1 H), 6.43 (d, J = 2.3 Hz, 1 H), 4.17 (q, J = 7.1 Hz, 2 H; CH₂), 3.91 (s, 2 H; CH₂), 3.86 (s, 3 H; OCH₃), 3.85 (s, 3 H; OCH₃), 1.23 (t, J = 7.1 Hz, 3 H; CH₃) ppm.

¹³C NMR (75 MHz, CDCl₃): δ = 191.2 (C=O), 168.5, 165.2, 161.1, 133.2 (CH), 119.5, 105.6 (CH), 98.0 (CH), 60.8 (CH₂), 55.5 (OCH₃), 55.2 (OCH₃), 50.6 (CH₂), 14.1 (CH₃) ppm.

The ¹H NMR spectral data are in accordance with the literature data.¹¹⁴

(*E*)-2-Ethoxycarbonyl-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)propen-1-one



(α -COOEt-TMC, **72**).⁵³ A solution of 3,4-dimethoxybenzaldehyde (**29**) (60.0 mg, 0.361 mmol, 1.0 eq.), β -keto ester **67** (100 mg, 0.396 mmol, 1.1 eq.), piperidine (3.60 μ L, 0.0361 mmol, 0.1 eq.) and glacial AcOH (10.4 μ L, 0.180 mmol, 0.5 eq.) in dry benzene (2 mL) was heated to reflux over 4 Å molecular sieves until no more change was observed by TLC (2 days). The reaction mixture was

cooled to room temperature and diluted with EtOAc (35 mL). This mixture was washed with 1 M aq. HCl (2 × 10 mL), 1 M aq. NaOH (2 × 10 mL) and brine (2 × 10 mL). The organic layer was dried with MgSO₄, filtered, and the solvent was evaporated *in vacuo*. The product was purified on preparative TLC plates (SiO₂, petroleum ether/EtOAc, 7:3) to afford α -COOEt-TMC (**72**) (103 mg, 0.257 mmol, 71%) as a pale yellow oil. The stereochemistry of

chalcone **72** was assigned as the *E* isomer based on the $^3J(\text{C},\text{H})$ coupling constants of $\text{C}_{\text{ketone},\text{H}}$ and $\text{C}_{\text{ester},\text{H}}$.⁵⁶ $^3J(\text{C},\text{H}) = 10.1$ Hz for $\text{C}_{\text{ketone},\text{H}}$; $^3J(\text{C},\text{H}) = 8.1$ Hz for $\text{C}_{\text{ester},\text{H}}$.

^1H NMR (300 MHz, CDCl_3): $\delta = 8.05$ (d, $J = 8.8$ Hz, 1 H), 7.54 (s, 1 H; $\beta\text{-H}$), 6.97 (dd, $J = 8.4, 2.0$ Hz, 1 H), 6.88 (d, $J = 2.1$ Hz, 1 H), 6.74 (d, $J = 8.4$ Hz, 1 H), 6.55 (dd, $J = 8.8, 2.3$ Hz, 1 H), 6.40 (d, $J = 2.3$ Hz, 1 H), 4.21 (q, $J = 7.1$ Hz, 2 H; CH_2), 3.85 (s, 3 H; OCH_3), 3.83 (s, 3 H; OCH_3), 3.77 (s, 3 H; OCH_3), 3.65 (s, 3 H; OCH_3), 1.17 (t, $J = 7.1$ Hz, 3 H; CH_3) ppm.

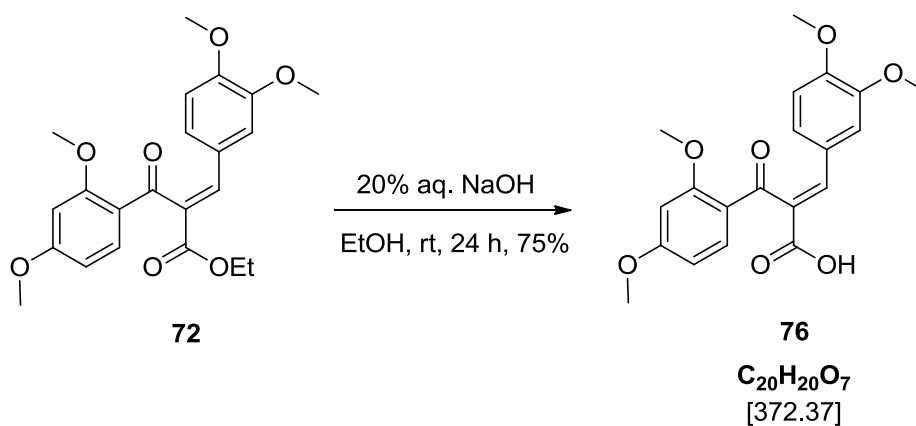
^{13}C NMR (75 MHz, CDCl_3): $\delta = 192.3$ ($\text{C}_{\text{ketone}=\text{O}}$), 165.6 ($\text{C}_{\text{ester}=\text{O}}$), 165.6, 162.0, 150.3, 148.6, 137.9 ($\beta\text{-CH}$), 133.6, 133.4 (CH), 126.4, 124.4 (CH), 120.1, 112.0 (CH), 110.8 (CH), 105.8 (CH), 98.5 (CH), 60.8 (CH_2), 55.8 (OCH_3), 55.6 (OCH_3), 55.6 (OCH_3), 55.5 (OCH_3), 14.2 (CH_3) ppm.

IR (neat): 2938, 2839, 1711, 1645, 1592, 1513, 1461, 1247, 1220, 1142, 1083, 1019 cm^{-1} .

MS (EI): m/z (%): 400 [M^{+}] (54), 354 (21), 299 (40), 165 (100).

HRMS (EI): calcd for $\text{C}_{22}\text{H}_{24}\text{O}_7$: 400.1522 [M^{+}]; found 400.1524.

6.2.5. Synthesis of (*E*)-2-(2,4-dimethoxybenzoyl)-3-(3,4-dimethoxyphenyl)propenoic acid ($\alpha\text{-COOH-TMC}$, **76**)⁵⁵



After dissolving the α -ester chalcone **72** (167 mg, 0.417 mmol, 1.0 eq.) in EtOH (3 mL), a 20% aqueous solution of NaOH (1.25 mL, 6.25 mmol, 15.0 eq.) was added and the mixture was stirred at room temperature for 24 h. Subsequently, brine (10 mL) was added to the reaction mixture and the solution was extracted with EtOAc (3×20 mL). The aqueous layer was acidified with 1 M aq. HCl to pH 1 and the mixture was extracted again with EtOAc (3×20 mL). The organic layers were combined, dried over MgSO_4 , filtered, and the solvent was

evaporated *in vacuo*. The product was purified on preparative TLC plates (SiO₂, CH₂Cl₂/EtOH/HCO₂H, 50:1:0.5) to afford α -acid chalcone **76** (116 mg, 0.312 mmol, 75%) as a yellow solid, which was recrystallized from EtOAc/petroleum ether; m.p. 133 °C; the same double bond configuration as for α -COOEt-TMC (**72**) is assumed which is *E*.

¹H NMR (400 MHz, CDCl₃): δ = 8.05 (d, *J* = 8.8 Hz, 1 H), 7.65 (s, 1 H; β -H), 6.99 (dd, *J* = 8.4, 1.9 Hz, 1 H), 6.89 (d, *J* = 1.9 Hz, 1 H), 6.75 (d, *J* = 8.4 Hz, 1 H), 6.55 (dd, *J* = 8.9, 2.2 Hz, 1 H), 6.40 (d, *J* = 2.2 Hz, 1 H), 3.85 (s, 3 H; OCH₃), 3.84 (s, 3 H; OCH₃), 3.78 (s, 3 H; OCH₃), 3.66 (s, 3 H; OCH₃) ppm.

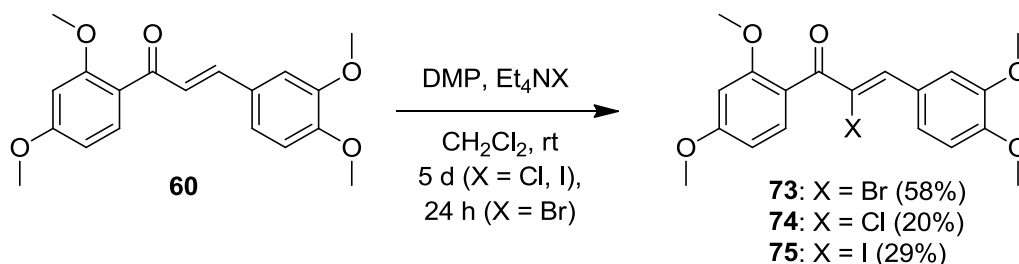
¹³C NMR (101 MHz, CDCl₃): δ = 192.0 (C_{ketone}=O), 170.3 (C_{acid}=O), 165.8, 162.2, 150.8, 148.7, 140.5 (β -CH), 133.5 (CH), 132.3, 126.2, 124.9 (CH), 119.9, 112.3 (CH), 110.8 (CH), 106.1 (CH), 98.6 (CH), 55.9 (OCH₃), 55.7 (OCH₃), 55.6 (2 OCH₃) ppm.

IR (neat): 2959, 2835, 1671, 1638, 1592, 1518, 1253, 1146, 1020 cm⁻¹.

MS (ESI⁺): *m/z* (%): 417.0 [M+HCOO]⁺ (47), 743.2 [2M-H]⁺ (100).

HRMS (ES-MS): calcd for C₂₀H₁₉O₇: 371.1136 [M-H]⁺; found 371.1147.

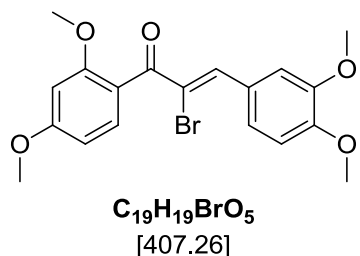
6.2.6. General procedure for the synthesis of α -halogen-chalcones (α -Br/Cl/I-TMCs, **73-75**)



The known procedure to prepare an α -bromochalcone⁴⁹ could be also applied to synthesize the α -chloro and α -iodo compounds. Et₄NX (X = Cl, Br, I) (1.2 or 2.0 eq.) was added in one portion to a stirred suspension of Dess-Martin periodinane (DMP, **19**) (1.2 or 2.0 eq.) in dry CH₂Cl₂, so that the color of the solution turned to orange-yellow. After 10 min of stirring at room temperature, chalcone **60** (1.0 eq.) was added and the reaction mixture stirred at the same temperature. The formed precipitate was filtered off and washed with CH₂Cl₂. The filtrate was washed with a saturated aqueous solution of NaHSO₃ or Na₂S₂O₅, followed by a

saturated aqueous solution of NaHCO₃, then H₂O. The organic layer was dried over MgSO₄ and then concentrated *in vacuo*. The products were purified on preparative TLC plates.

(Z)-2-Bromo-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxy-phenyl)prop-2-en-1-one (α-Br-TMC, 73).



The α-bromochalcone **73** was prepared according to the general procedure (5.2.2) starting from Et₄NBr (230 mg, 1.10 mmol, 1.2 eq.) and DMP (**19**) (465 mg, 1.10 mmol, 1.2 eq.) in dry CH₂Cl₂ (2 mL). α-H-TMC (**60**) (300 mg, 0.914 mmol, 1.0 eq.) was added and the reaction mixture stirred for 24 h.⁴⁹

The product was purified first on preparative TLC plates (SiO₂, petroleum ether/EtOAc, 7:3) and then recrystallized from EtOAc/petroleum ether to afford yellow crystals of α-Br-TMC (**73**) (216 mg, 0.530 mmol, 58%) as the pure *Z* isomer; m.p. 125 °C.

¹H NMR (400 MHz, CDCl₃): δ = 7.67 (d, *J* = 2.0 Hz, 1 H), 7.65 (s, 1 H; β-H), 7.39-7.33 (m, 2 H), 6.88 (d, *J* = 8.5 Hz, 1 H), 6.55 (dd, *J* = 8.4, 2.3 Hz, 1 H), 6.50 (d, *J* = 2.2 Hz, 1 H), 3.92 (s, 6 H; 2 OCH₃), 3.87 (s, 3 H; OCH₃), 3.79 (s, 3 H; OCH₃) ppm.

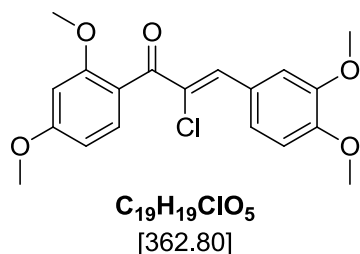
¹³C NMR (101 MHz, CDCl₃): δ = 190.4 (C=O), 163.2, 159.0, 151.0, 148.5, 142.3 (β-CH), 131.4 (CH), 126.7, 125.4 (CH), 122.6, 120.5, 112.7 (CH), 110.6 (CH), 104.7 (CH), 98.9 (CH), 56.0 (OCH₃), 55.9 (OCH₃), 55.8 (OCH₃), 55.5 (OCH₃) ppm.

IR (neat): 1637, 1602, 1589, 1511, 1259, 1247, 1212, 1145, 1018, 819 cm⁻¹.

MS (EI): *m/z* (%): 406 [M⁺] (14), 327 (49), 165 (100).

HRMS (EI): calcd for C₁₉H₁₉BrO₅: 406.0416 [M⁺]; found 406.0423.

(Z)-2-Chloro-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxy-phenyl)prop-2-en-1-one (α-Cl-TMC, 74).



The α-chlorochalcone **74** was prepared according to the general procedure starting (5.2.2) from Et₄NCl (510 mg, 3.05 mmol, 2.0 eq.) and DMP (1.29 g, 3.05 mmol, 2.0 eq.) in dry CH₂Cl₂ (3.5 mL). α-H-TMC (**60**) (500 mg, 1.53 mmol, 1.0 eq.) was added and the reaction mixture was stirred, until no more change was observed by TLC (5 days). The product was purified first on preparative TLC plates (SiO₂, petroleum ether/EtOAc, 7:3) and then recrystallized from EtOAc/petroleum

ether to afford yellow crystals of α -Cl-TMC (**74**) (108 mg, 0.298 mol, 20%) as the pure *Z* isomer; m.p. 119 °C.

^1H NMR (400 MHz, CDCl_3): δ = 7.58 (d, J = 2.0 Hz, 1 H), 7.40 (s, 1 H; β -H), 7.39-7.33 (m, 2 H), 6.89 (d, J = 8.5 Hz, 1 H), 6.56 (dd, J = 8.4, 2.2 Hz, 1 H), 6.51 (d, J = 2.2 Hz, 1 H), 3.93 (s, H, OCH_3), 3.92 (s, 3 H; OCH_3), 3.88 (s, 3 H; OCH_3), 3.80 (s, 3 H; OCH_3) ppm.

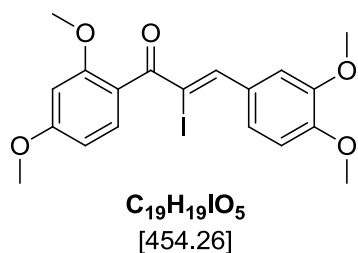
^{13}C NMR (101 MHz, CDCl_3): δ = 190.4 (C=O), 163.3, 159.1, 151.0, 148.7, 138.9 (β -CH), 131.3 (CH), 130.1, 126.2 (CH), 125.6, 120.7, 113.1 (CH), 110.8 (CH), 104.7 (CH), 98.9 (CH), 56.0 (OCH_3), 56.0 (OCH_3), 55.8 (OCH_3), 55.6 (OCH_3) ppm.

IR (neat): 1639, 1600, 1592, 1513, 1260, 1248, 1146, 1020, 821 cm^{-1} .

MS (ESI^+): m/z (%): 363 [$\text{M}+\text{H}$] $^+$ (100).

HRMS (ES-MS): calcd for $\text{C}_{19}\text{H}_{20}\text{ClO}_5$: 363.0994 [$\text{M}+\text{H}$] $^+$; found 363.0997.

(*Z/E*)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-iodoprop-2-en-1-one (α -I-



TMC, **75**). The α -iodochalcone **75** was prepared according to the general procedure (5.2.2) starting from Et_4NI (784 mg, 3.05 mmol, 2.0 eq.) and DMP (1.29 g, 3.05 mmol, 2.0 eq.) in dry CH_2Cl_2 (3.5 mL). α -H-TMC (**60**) (500 mg, 1.53 mmol, 1.0 eq.) was added and the reaction mixture was stirred until no more

change was observed by TLC (5 days). The product was purified on preparative TLC plates to afford the yellow solid α -I-TMC (**75**) (200 mg, 0.440 mmol, 29%) as a *Z/E* mixture of 93:7; m.p. 97 °C.

***Z*-75:** **^1H NMR** (400 MHz, CDCl_3): δ = 7.67 (d, J = 1.9 Hz, 1 H), 7.64 (s, 1 H; β -H), 7.33 (d, J = 8.4 Hz, 1 H), 7.29 (dd, J = 8.5, 1.9 Hz, 1 H), 6.87 (d, J = 8.4 Hz, 1 H), 6.52 (dd, J = 8.4, 2.2 Hz, 1 H), 6.48 (d, J = 2.2 Hz, 1 H), 3.91 (s, 3 H; OCH_3), 3.90 (s, 3 H; OCH_3), 3.84 (s, 3 H; OCH_3), 3.77 (s, 3 H; OCH_3) ppm.

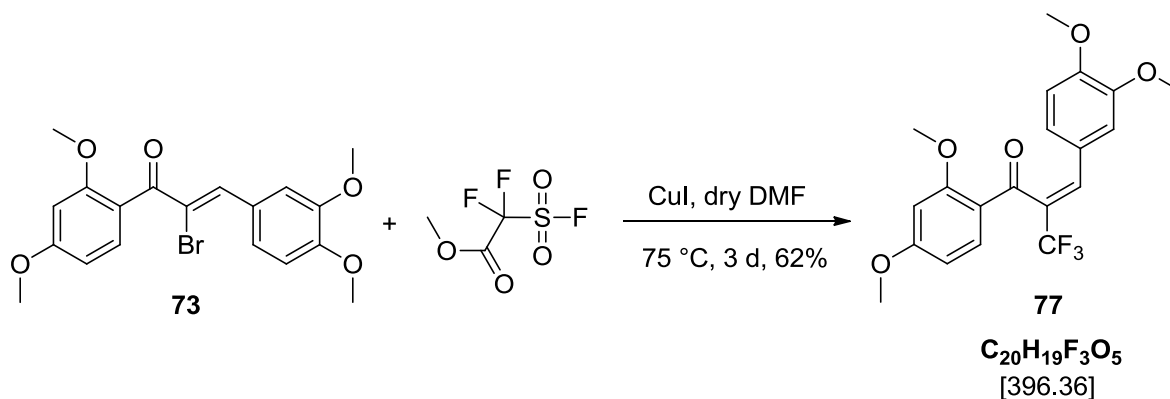
^{13}C NMR (101 MHz, CDCl_3): δ = 191.8 (C=O), 163.3, 159.0, 150.9, 148.6 (β -CH), 148.3, 131.6 (CH), 127.7, 124.9 (CH), 119.6, 112.0 (CH), 110.5 (CH), 104.7 (CH), 104.2, 98.9 (CH), 56.0 (OCH_3), 55.9 (OCH_3), 55.8 (OCH_3), 55.5 (OCH_3) ppm.

IR (neat): 1631, 1598, 1510, 1259, 1231, 1212, 1144, 1017, 817 cm^{-1} .

MS (ESI⁺): m/z (%): 455 [M+H]⁺ (100).

HRMS (ES-MS): calcd for C₁₉H₂₀IO₅: 455.0350 [M+H]⁺; found 455.0347.

6.2.7. Synthesis of (*E*)-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-(trifluoromethyl)prop-2-en-1-one (α -CF₃-TMC, **77**)⁸³



A mixture of α -Br-TMC (**73**) (200 mg, 0.491 mmol, 1.0 eq.), CuI (112 mg, 0.589 mmol, 1.2 eq.) and methyl fluorosulfonyldifluoroacetate (MFSDA) (456 μ L, 3.58 mmol, 7.3 eq.) in dry DMF (20 mL) was stirred at 75 °C under a N₂ atmosphere for 3 days. Upon completion of the reaction, the mixture was diluted with Et₂O (40 mL) and filtered. The solution was poured into H₂O (40 mL) and the mixture extracted with Et₂O (4 \times 40 mL). The combined extracts were washed with H₂O (3 \times 5 mL), dried over MgSO₄, filtered, and concentrated *in vacuo*. The product was purified on preparative TLC plates (SiO₂, CHCl₃/MeOH, 99:1) to afford α -CF₃-TMC (**77**) (120 mg, 0.303 mmol, 62 %) as a pale yellow oil. The stereochemistry of chalcone **77** was assigned as the *E* isomer based on its ¹⁹F NMR shift.⁸⁴

¹H NMR (400 MHz, CDCl₃): δ = 7.82 (d, J = 8.8 Hz, 1 H), 7.11 (d, J = 1.4 Hz, 1 H; β -H), 6.86 (dd, J = 8.3, 2.0 Hz, 1 H), 6.78 (d, J = 2.0 Hz, 1 H), 6.70 (d, J = 8.4 Hz, 1 H), 6.44 (dd, J = 8.8, 2.3 Hz, 1 H), 6.34 (d, J = 2.3 Hz, 1 H), 3.80 (s, 6 H; 2 OCH₃), 3.77 (s, 3 H; OCH₃), 3.64 (s, 3 H; OCH₃) ppm.

¹³C NMR (101 MHz, CDCl₃): δ = 189.7 (C=O), 165.7, 162.0, 150.1, 148.5, 134.2 (CH), 133.8 (q, J = 5.9 Hz, β -CH), 130.2 (q, J = 29.3 Hz, α -C), 125.4, 123.4 (CH), 122.8 (q, J = 274.0 Hz, CF₃), 119.6, 111.7 (CH), 110.7 (CH), 105.5 (CH), 98.5 (CH), 55.7 (OCH₃), 55.5 (OCH₃), 55.49 (OCH₃), 55.48 (OCH₃) ppm.

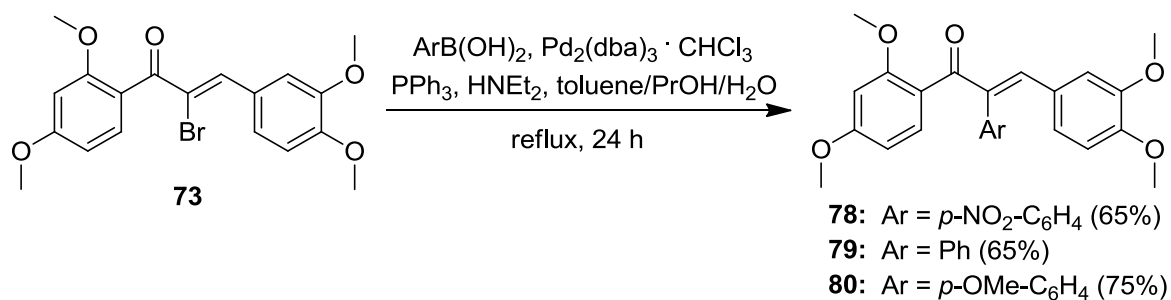
¹⁹F NMR (376 MHz, CDCl₃): δ = -62.1 (d, J = 1.1 Hz) ppm.

IR (neat): 3005, 2939, 2842, 1642, 1593, 1515, 1252, 1111, 1021 cm^{-1} .

MS (ESI^+): m/z (%): 397 $[\text{M}+\text{H}]^+$ (100).

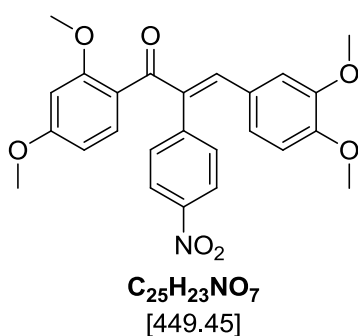
HRMS (ES-MS): calcd for $\text{C}_{20}\text{H}_{20}\text{F}_3\text{O}_5$: 397.1257 $[\text{M}+\text{H}]^+$; found 397.1265.

6.2.8. General procedure for synthesis of α -Ar-TMCs 78-80⁴⁸



A mixture of α -Br-TMC (**73**) (150 mg, 0.368 mmol, 1.0 eq.), the corresponding boronic acid (0.442 mmol, 1.2 eq.), $[\text{Pd}_2(\text{dba})_3] \cdot \text{CHCl}_3$ (8.0 mg, 0.0080 mmol, 0.022 eq.), and PPh_3 (4.0 mg, 0.015 mmol, 0.042 eq.) was dissolved in toluene (2.2 mL) and *n*-PrOH (0.75 mL) and degassed. After stirring for 10 min, HNEt_2 (50 μL , 0.478 mmol, 1.3 eq.) and H_2O (0.60 mL) were added, the mixture was degassed again and then stirred under reflux for 24 h. After cooling to room temperature, it was poured into EtOAc (50 mL). The organic layer was washed with 0.2 M aq. NaOH (25 mL), 0.05 M aq. HCl (25 mL), and H_2O (2×25 mL), dried over MgSO_4 , and filtered. The solvent was removed *in vacuo* and the product was purified on preparative TLC plates (SiO_2 , EtOAc/petroleum ether 3:7) to afford *E*- α -aryl-chalcones. The pure isomers from preparative TLC isomerized upon handling and analysis in solution to *E/Z* mixtures. The configuration of the double bond was assigned based on literature data to be the same as for starting chalcone **73**.⁴⁸

(*E*)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-(4-nitrophenyl)prop-2-en-1-one



(α -*p*-NO₂-C₆H₄-TMC, **78**). Yellow solid (65%, *E* isomer); m.p. 59 °C; the *E/Z* ratio observed later for the isomerized product in CDCl_3 is 4:1.

***E*-78**: ¹H NMR (300 MHz, CDCl_3): δ = 8.24-8.18 (m, 2 H), 7.48-7.43 (m, 3 H), 7.32 (s, 1 H; β -H), 6.73-6.64 (m, 2 H), 6.56 (dd, J = 8.5, 2.2 Hz, 1 H), 6.44 (dd, J = 5.5, 1.9 Hz, 2 H), 3.86

(s, 3 H; OCH₃), 3.84 (s, 3 H; OCH₃), 3.74 (s, 3 H; OCH₃), 3.49 (s, 3 H; OCH₃) ppm.

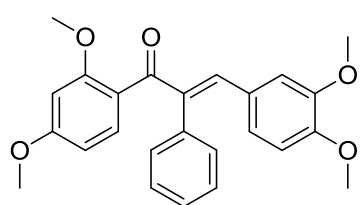
E-78: ¹³C NMR (75 MHz, CDCl₃): δ = 195.7 (C=O), 163.3, 158.9, 150.4, 148.5, 147.1, 144.6, 142.1 (β -CH), 138.5, 131.5 (CH), 131.4 (2 CH), 126.9, 124.8 (CH), 123.5 (2 CH), 121.8, 112.7 (CH), 110.7 (CH), 104.8 (CH), 98.6 (CH), 55.8 (OCH₃), 55.6 (OCH₃), 55.5 (OCH₃), 55.3 (OCH₃) ppm.

IR (neat): 2936, 2837, 1640, 1593, 1511, 1459, 1341, 1259, 1208, 1022 cm⁻¹.

MS (EI): m/z (%): 449 [M⁺] (47), 311 (24), 165 (91).

HRMS (EI): calcd for C₂₅H₂₃NO₇: 449.1475 [M⁺]; found 449.1482.

(E)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-phenylprop-2-en-1-one (α -Ph-



C₂₅H₂₄O₅
[404.46]

TMC, 79). Yellow solid (65%, *E*); m.p. 50 °C; the *E/Z* ratio observed later for the isomerized product in CDCl₃ is 3:2.

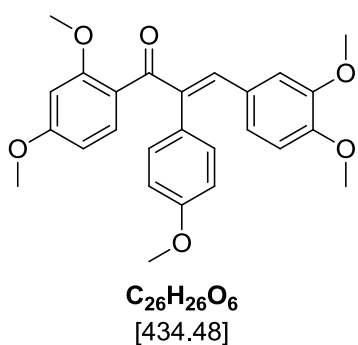
E-79: ¹H NMR (400 MHz, CDCl₃): δ = 7.42 (d, *J* = 8.4 Hz, 1 H), 7.39-7.35 (m, 2 H), 7.31-7.27 (m, 3 H), 7.23 (s, 1 H; β -H), 6.76-6.68 (m, 2 H), 6.53 (dd, *J* = 8.4, 2.2 Hz, 1 H), 6.44 (dd, *J* = 9.6, 2.1 Hz, 2 H), 3.83 (s, 3 H; OCH₃), 3.82 (s, 3 H; OCH₃), 3.74 (s, 3 H; OCH₃), 3.39 (s, 3 H; OCH₃) ppm.

E-79: ¹³C NMR (101 MHz, CDCl₃): δ = 196.8 (C=O), 162.7, 158.8, 149.8, 148.1, 140.8 (β -CH), 140.5, 137.0, 131.2 (CH), 130.1 (2 CH), 128.5 (2 CH), 127.8, 127.4 (CH), 125.2 (CH), 122.6, 112.3 (CH), 110.3 (CH), 104.4 (CH), 98.6 (CH), 55.7 (OCH₃), 55.6 (OCH₃), 55.4 (OCH₃), 55.0 (OCH₃) ppm.

IR (neat): 2934, 2837, 1642, 1593, 1512, 1261, 1245, 1207, 1141, 1020 cm⁻¹.

MS (ESI⁺): m/z (%): 405 [M+H]⁺ (100).

HRMS (ES-MS): calcd for C₂₅H₂₅O₅: 405.1697 [M+H]⁺; found 405.1701.

(E)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-(4-methoxyphenyl)prop-2-en-1-

one (α -*p*-OMe-C₆H₄-TMC, 80). Yellow solid (75%, *E*); m.p. 45 °C; the *E/Z* ratio observed later for the isomerized product in CDCl₃ is 8:1.

***E*-80:** ¹H NMR (300 MHz, CDCl₃): δ = 7.41 (d, *J* = 8.4 Hz, 1 H), 7.21-7.16 (m, 3 H), 6.93-6.88 (m, 2 H), 6.77-6.68 (m, 2 H), 6.54-6.50 (m, 2 H), 6.42 (d, *J* = 2.2 Hz, 1 H), 3.84 (s, 3 H; OCH₃), 3.83 (s, 3 H; OCH₃), 3.80 (s, 3 H; OCH₃), 3.74 (s, 3 H; OCH₃), 3.47 (s, 3 H; OCH₃) ppm.

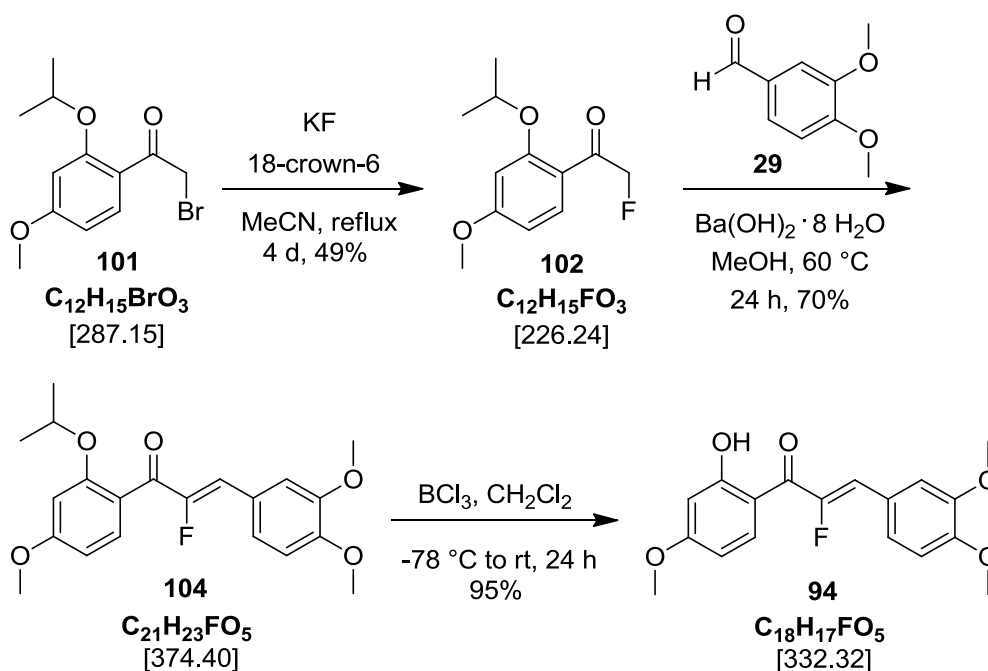
***E*-80:** ¹³C NMR (75 MHz, CDCl₃): δ = 197.3 (C=O), 162.7, 159.0, 158.9, 149.7, 148.1, 140.5 (β -CH), 140.3, 131.3 (2 (CH)), 131.2 (CH), 129.1, 128.1, 125.0 (CH), 122.8, 114.0 (2 CH), 112.5 (CH), 110.4 (CH), 104.4 (CH), 98.6 (CH), 55.7 (OCH₃), 55.6 (OCH₃), 55.4 (OCH₃), 55.2 (OCH₃), 55.2 (OCH₃) ppm.

IR (neat): 2939, 2835, 1638, 1595, 1575, 1508, 1460, 1241, 1207, 1127, 1020 cm⁻¹.

MS (ESI⁺): *m/z* (%): 435 [M+H]⁺ (100).

HRMS (ES-MS): calcd for C₂₆H₂₇O₆: 435.1802 [M+H]⁺; found 435.1800.

6.2.9. Synthesis of (Z)-3-(3,4-dimethoxyphenyl)-2-fluoro-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one (α -F-HC, **94**)



2-Bromo-1-(2-isopropoxy-4-methoxyphenyl)ethanone (101) was prepared by Martin Wild in our group by the same procedure used for preparation of 2-bromo-1-(2,4-dimethoxyphenyl)ethanone (**81**).

2-Fluoro-1-(2-isopropoxy-4-methoxyphenyl)ethanone (102).¹³ To freshly dried KF (1.79 g, 30.9 mmol, 17.8 eq.), 2-bromoacetophenone **101** (500 mg, 1.74 mmol, 1.0 eq.) and 18-crown-6 (92.0 mg, 0.348 mmol, 0.2 eq.) in dry MeCN (10 mL) were added under N_2 atmosphere. The mixture was refluxed for 4 days. The solvent was then removed *in vacuo* and the residue partitioned between CH_2Cl_2 and H_2O . The organic fraction was dried and evaporated. The residue was subjected to chromatography on preparative TLC plates (SiO_2 , petroleum ether/EtOAc, 7:3) yielded 2-fluoroacetophenone **102** as a white solid (195 mg, 0.862 mmol, 49%); m.p. 72 °C.

1H NMR (400 MHz, $CDCl_3$): δ = 8.05 (d, J = 8.9 Hz, 1 H), 6.56 (dd, J = 8.9, 2.3 Hz, 1 H), 6.42 (d, J = 2.2 Hz, 1 H), 5.40 (d, J = 48.2 Hz, 2 H; CH_2F), 4.69 (sept, J = 6.1 Hz, 1 H; $CH(CH_3)_2$), 3.86 (s, 3 H; OCH_3), 1.43 (d, J = 6.1 Hz, 6 H; 2 CH_3) ppm.

^{13}C NMR (75 MHz, CDCl_3): δ = 192.2 (d, J = 14.1 Hz, $\text{CH}_2\text{F}-\text{C}=\text{O}$), 165.4, 159.7, 133.1 (d, J = 2.5 Hz, $\text{FCH}_2-\text{C}(\text{O})-\text{C}$), 117.9, 105.8 (CH), 99.3 (CH), 86.2 (d, J = 177.8 Hz, CH_2F), 71.0 ($\text{CH}(\text{CH}_3)_2$), 55.6 (OCH_3), 22.0 (2 CH_3) ppm.

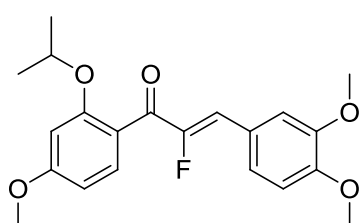
^{19}F -NMR (376 MHz, CDCl_3): δ = -222.9 (t, J = 49 Hz, 1 F) ppm.

IR (neat): 1662, 1603, 1286, 1259, 1209, 1073, 990, 832 cm^{-1} .

MS (EI): m/z (%): 226 [M^{+}] (7), 193 [$\text{M}-\text{CH}_2\text{F}$] (7), 151 (100).

HRMS (EI): calcd. for $\text{C}_{12}\text{H}_{15}\text{FO}_3$: 226.1205 [M^{+}]; found 226.1007.

(Z)-3-(3,4-Dimethoxyphenyl)-2-fluoro-1-(2-isopropoxy-4-methoxyphenyl)prop-2-en-1-



one (α -F-pHC, **104).** The α -fluorochalcone **104** was prepared according to the general procedure (5.2.1) starting from 2-fluoroacetophenone **102** (195 mg, 0.862 mmol, 1.0 eq.) and 3,4-dimethoxybenzaldehyde (**29**) (143 mg, 0.862 mmol, 1.0 eq.) which were dissolved in dry MeOH under N_2 atmosphere.

The suspension was stirred at 60 $^{\circ}\text{C}$ for 24 h. Purification on preparative TLC plates (SiO_2 , petroleum ether/EtOAc, 7:3) afforded the yellow solid α -F-pHC (**104**) (225 mg, 0.601 mmol, 70%) which was recrystallized from EtOAc/petroleum ether; m.p. 95 $^{\circ}\text{C}$.

^1H NMR (400 MHz, CDCl_3): δ = 7.42 (d, J = 8.5 Hz, 1 H), 7.29 (bs, 1 H), 7.20 (dd, J = 8.4, 1.9 Hz, 1 H), 6.88 (d, J = 8.4 Hz, 1 H), 6.63 (d, J = 35.8 Hz, 1 H; β -H), 6.54 (dd, J = 8.5, 2.2 Hz, 1 H), 6.48 (d, J = 2.2 Hz, 1 H), 4.56 (sept, J = 6.1 Hz, 1 H; $\text{CH}(\text{CH}_3)_2$), 3.92 (s, 3 H; OCH_3), 3.89 (s, 3 H; OCH_3), 3.85 (s, 3 H; OCH_3), 1.28 (d, J = 6.0 Hz, 6 H; 2 CH_3) ppm.

^{13}C NMR (101 MHz, CDCl_3): δ = 188.2 (d, J = 30.9 Hz, $\text{C}=\text{O}$), 163.5, 157.8, 154.4 (d, J = 268.1 Hz, α -C), 150.3 (d, J = 3.2 Hz), 148.9, 131.6 (CH), 124.8 (d, J = 4.1 Hz), 124.4 (d, J = 7.5 Hz, CH), 121.2, 118.1 (d, J = 6.4 Hz, β -CH), 112.6 (d, J = 10.1 Hz, CH), 111.0 (CH), 104.8 (CH), 100.4 (CH), 70.8 ($\text{CH}(\text{CH}_3)_2$), 55.8 (OCH_3), 55.8 (OCH_3), 55.8 (OCH_3), 55.4 (OCH_3) ppm.

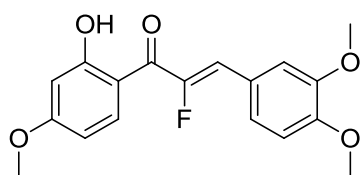
^{19}F NMR (376 MHz, CDCl_3): δ = -123.4 (d, J = 35.8 Hz, 1 F) ppm.

IR (neat): 2976, 2932, 2845, 1597, 1578, 1512, 1447, 1420, 1300, 1264, 1205, 1143, 1019, 988, 828 cm^{-1} .

MS (ESI⁺): *m/z* (%): 375 [M+H]⁺ (100).

HRMS (ES-MS): calcd. for C₂₁H₂₄FO₅: 375.1602 [M+H]⁺; found 375.1605.

(Z)-3-(3,4-Dimethoxyphenyl)-2-fluoro-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one



(α-F-HC, **94):** To a solution of α-F-pHC (**104**) (164 mg, 0.438 mmol, 1.0 eq.) in dry CH₂Cl₂ (5 mL) was added at –78 °C dropwise a 1 M solution of BCl₃ in hexane (1.31 mL, 1.31 mmol, 3.0 eq.). Upon addition the yellow solution turned

immediately deep-red. After 30 min at –78 °C the solution was allowed to warm to 0 °C and the solution was stirred for about 3 h at this temperature then it was left overnight to stir at room temperature. EtOH (1 mL) and H₂O (15 mL) were added to the reaction mixture and the resulting mixture extracted with CH₂Cl₂ (3 × 15 mL). The combined organic layers were dried over MgSO₄, filtered and the solvent removed under reduced pressure. Purification was performed on preparative TLC plates (SiO₂, petroleum ether/EtOAc, 7:3) then the product was recrystallized from EtOAc/petroleum ether to get α-F-HC (**94**) as a yellow solid (120 mg, 0.361 mmol, 82%); m.p. 112 °C.

¹H NMR (400 MHz, CDCl₃): δ = 12.72 (s, 1H, 2'-OH), 8.07-8.00 (m, 1 H), 7.39-7.26 (m, 2 H), 6.93 (d, *J* = 37.6 Hz, 1 H; β-H), 6.91 (d, *J* = 8.4 Hz, 1 H), 6.50-6.74 (m, 2 H), 3.93 (s, 6 H; 2 OCH₃), 3.87 (s, 3 H; OCH₃) ppm.

¹³C NMR (101 MHz, CDCl₃): δ = 187.5 (d, *J* = 27.0 Hz, C=O), 167.0, 166.5 (d, *J* = 1.7 Hz), 154.3 (d, *J* = 271.4 Hz, α-C), 150.7 (d, *J* = 3.4 Hz), 149.0, 133.0 (d, *J* = 18.0 Hz, CH), 124.9 (d, *J* = 7.6 Hz, CH), 124.5 (d, *J* = 3.6 Hz), 119.0 (d, *J* = 5.4 Hz, β-CH), 112.9 (d, *J* = 9.9 Hz, CH), 112.3 (d, *J* = 3.8 Hz), 111.1 (CH), 108.0 (d, *J* = 2.8 Hz, CH), 101.1 (CH), 56.0 (OCH₃), 55.9 (OCH₃), 55.6 (OCH₃) ppm.

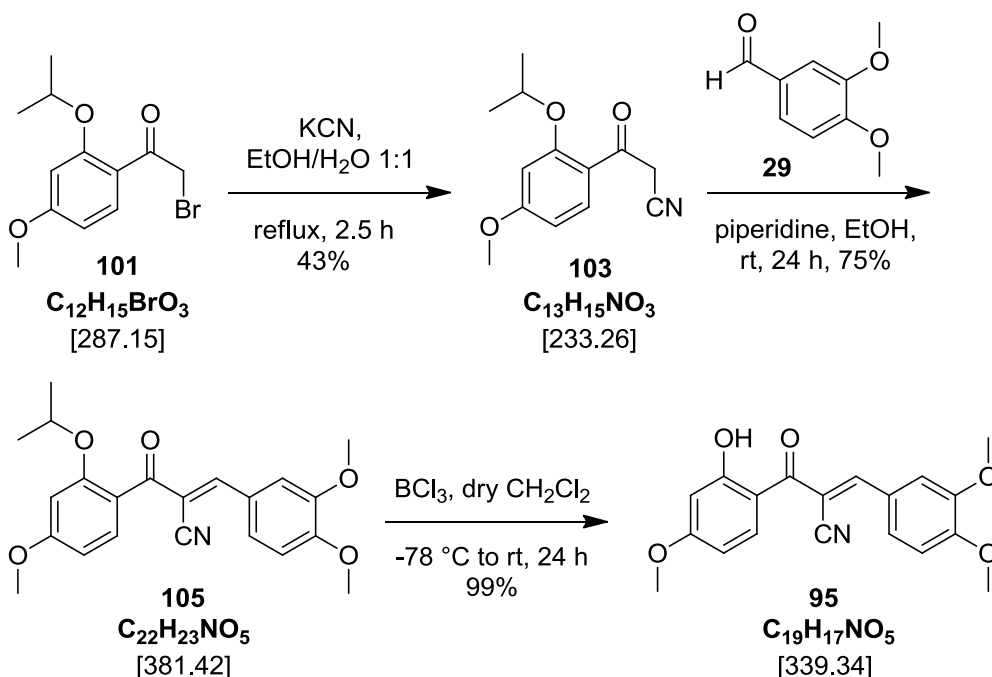
¹⁹F NMR (376 MHz, CDCl₃): δ = -119.9 (d, *J* = 37.5 Hz, 1 F) ppm.

IR (neat): 3376, 2952, 2845, 1625, 1569, 1513, 1355, 1253, 1142, 1022, 966, 796 cm⁻¹.

MS (ESI⁺): *m/z* (%): 333 [M+H]⁺ (100).

HRMS (ES-MS): calcd. for C₁₈H₁₈FO₅: 333.1133 [M+H]⁺; found 333.1132.

6.2.10 Synthesis of (*E*)-2-cyano-1-(3,4-dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one (α -CN-HC, **95**)



3-(2-Isopropoxy-4-methoxyphenyl)-3-oxopropanenitrile (103). KCN (567 mg, 8.71 mmol, 5.0 eq.) in H₂O (1.7 mL) was added to a solution of 2-bromoacetophenone **101** (300 mg, 1.40 mmol, 1.0 eq.) in EtOH (1.7 mL). The resulting mixture was gradually heated to reflux (2.5 h). After cooling to room temperature, the reaction mixture was diluted with H₂O (20 mL), acidified with 1 M aq. HCl (pH 6), extracted with CH₂Cl₂ (8 × 50 mL), the organic extract was dried over MgSO₄ and the solvent evaporated under reduced pressure. The product was purified on preparative TLC plates (petroleum ether/EtOAc, 7:1) to afford 2-cyanoacetophenone **103** (175 mg, 0.751 mmol, 43%) as a beige solid; m.p. 99 °C.

¹H NMR (300 MHz, CDCl₃): δ = 7.92 (d, J = 8.9 Hz, 1 H), 6.55 (dd, J = 8.9, 2.3 Hz, 1 H), 6.43 (d, J = 2.2 Hz, 1 H), 4.71 (sept., J = 6.1 Hz, 1 H; CH(CH₃)₂), 4.04 (s, 2 H; CH₂CN), 3.86 (s, 3 H; OCH₃), 1.46 (d, J = 6.1 Hz, 6 H; 2 CH₃) ppm.

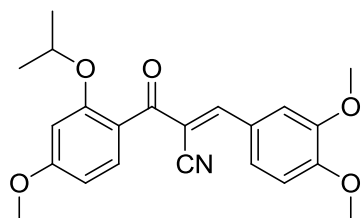
¹³C NMR (101 MHz, CDCl₃): δ = 186.2 (C=O), 165.8, 159.5, 133.8 (CH), 118.4, 115.0 (CN), 105.7 (CH), 99.5 (CH), 71.2, 55.7 (2 OCH₃), 34.1 (CH₂CN), 21.9 (2 CH₃) ppm.

IR (neat): 2966, 2926, 2852, 2261, 1660, 1597, 1570, 1440, 1379, 1320, 1280, 1253, 1202, 1105, 978, 929, 840 cm⁻¹.

MS (EI): m/z (%) = 233 (9.8) [M^{+}], 218 (6.8) [$M^{+}-CH_3$], 251 (100).

HRMS (EI): calcd. for $C_{13}H_{15}NO_3$: 233.1052 [M^{+}]; found 233.1053.

(E)-2-Cyano-3-(3,4-dimethoxyphenyl)-1-(2-isopropoxy-4-methoxyphenyl)prop-2-en-1-



one (α -CN-pHC, **105).**¹³ The 2-cyanoacetophenone **103** (150

mg, 0.643 mmol, 1.0 eq.) and 3,4-dimethoxybenzaldehyde (**29**) (107 mg, 0.643 mmol, 1.0 eq.) were warmed in EtOH (66 mL)

using the heating gun until they dissolved. The solution was

then cooled to room temperature and piperidine (100 μ L, 1.01 mmol, 2.1 eq.) was added. After 24 h of stirring at this temperature, the EtOH was removed under vacuum and the residue partitioned between H_2O (40 mL) and CH_2Cl_2 (3×50 mL). The CH_2Cl_2 fractions were dried over $MgSO_4$ and evaporated. The product was purified on preparative TLC plates (petroleum ether/EtOAc, 7:3) to afford α -CN-pHC (**105**) (172 mg, 0.451 mmol, 70%) as a yellow solid; m.p. 142 $^{\circ}C$.

1H NMR (400 MHz, $CDCl_3$): δ = 7.85 (s, 1 H, β -H), 7.81 (d, J = 2.0, 1 H), 7.49-7.45 (m, 2 H), 6.94 (d, J = 8.5 Hz, 1 H), 6.54 (dd, J = 8.6, 2.2 Hz, 1 H), 6.46 (d, J = 2.1 Hz, 1 H), 4.58 (sept, J = 6.0 Hz, 1 H; $CH(CH_3)_2$), 3.96 (s, 3 H; OCH_3), 3.95 (s, 3 H; OCH_3), 3.85 (s, 3 H; OCH_3), 1.31 (d, J = 6.0 Hz, 6 H; 2 CH_3) ppm.

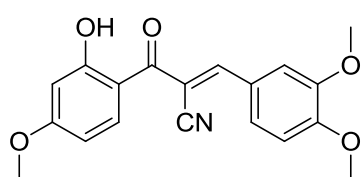
^{13}C NMR (101 MHz, $CDCl_3$): δ = 189.1 (C=O), 164.2, 157.9, 153.1, 152.1 (β -CH), 149.2, 132.3 (CH), 127.2 (CH), 125.3, 120.9, 118.0, 111.6 (CH), 111.0 (CH), 110.3, 105.1 (CH), 99.9 (CH), 71.0 ($CH(CH_3)_2$), 56.1 (OCH_3), 56.0 (OCH_3), 55.5 (OCH_3), 21.7 (2 CH_3) ppm.

IR (neat): 2972, 2200, 1662, 1600, 1565, 1506, 1422, 1265, 1167, 1143, 1116, 1013, 981, 822 cm^{-1} .

MS (ESI⁺) m/z (%): 382 [$M+H$]⁺ (100).

HRMS (ES-MS): calcd. for $C_{22}H_{24}NO_5$: 382.1649 [$M+H$]⁺; found 382.1648.

(E)-2-Cyano-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one



(α -CN-HC, **95):** To a solution of α -CN-pHC (**105**) (144 mg,

0.378 mmol, 1.0 eq.) in dry CH_2Cl_2 (6 mL) was added at

$-78^{\circ}C$ dropwise a 1 M solution of BCl_3 in hexane (1.11 mL, 1.11 mmol, 3.0 eq.). Upon addition the yellow solution turned

immediately deep-red. After 30 min at $-78\text{ }^{\circ}\text{C}$ the solution was allowed to warm to $0\text{ }^{\circ}\text{C}$ and the solution was stirred for about 3 h at this temperature then it was left overnight to stir at RT. Then 1.5 mL EtOH and 40 mL H_2O were added to the reaction mixture and the resulting mixture extracted with CH_2Cl_2 ($3 \times 50\text{ mL}$). The combined organic layers were dried over MgSO_4 , filtered and the solvent removed under reduced pressure. Purification by recrystallization from EtOAc/petroleum ether afforded $\alpha\text{-CN-HC}$ (**95**) as a yellow solid (120 mg, 0.354 mmol, 94%); m.p. $183\text{ }^{\circ}\text{C}$.

^1H NMR (400 MHz, CDCl_3): δ = 12.06 (s, 1 H; $2'\text{-OH}$), 7.99 (dd, J = 7.8, 1.6 Hz, 1 H), 7.89 (s, 1 H; $\beta\text{-H}$), 7.85 (d, J = 2.1, 1 H), 7.48 (dd, J = 8.4, 2.1 Hz, 1 H), 6.96 (d, J = 8.5 Hz, 1 H), 6.53-6.50 (m, 2 H), 3.98 (s, 3 H; OCH_3), 3.97 (s, 3 H; OCH_3), 3.87 (s, 3 H; OCH_3) ppm.

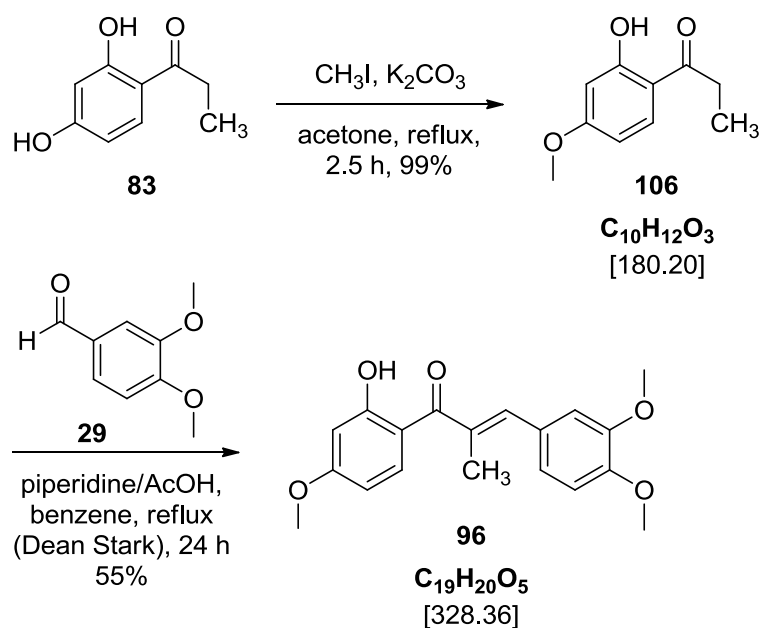
^{13}C NMR (101 MHz, CDCl_3): δ = 190.2 (C=O), 166.7, 166.2, 154.5 ($\beta\text{-CH}$), 153.6, 149.3, 132.9 (CH), 127.8 (CH), 125.1, 117.8, 112.2, 111.5 (CH), 111.0 (CH), 107.9, 105.8, 101.4(CH), 56.2 (OCH_3), 56.1 (OCH_3), 55.7 (OCH_3) ppm.

IR (neat): 2199, 1630, 1600, 1579, 1511, 1343, 1262, 1212, 1145, 1019, 842 cm^{-1} .

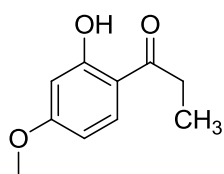
MS (ESI^+): m/z (%): 340 $[\text{M}+\text{H}]^+$ (100).

HRMS (ES-MS): calcd. for $\text{C}_{19}\text{H}_{18}\text{NO}_5$: 340.1179 $[\text{M}+\text{H}]^+$; found 340.1179.

6.2.11 Synthesis of (*E*)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)-2-methylprop-2-en-1-one ($\alpha\text{-Me-HC}$, **96**)



1-(2-Hydroxy-4-methoxyphenyl)propan-1-one (106).⁷¹ Anhydrous K₂CO₃ (912 mg, 6.60 mmol, 1.1 eq.) was added to a stirred solution of 1-(2,4-dihydroxyphenyl)-



ethanone (**83**) (1.00 g, 6.00 mmol, 1.0 eq.) in dry acetone (20 mL). After a few minutes, CH₃I (2.25 mL, 36.0 mmol, 6.0 eq.) was added to the suspension. The reaction mixture was refluxed for 2.5 h, cooled, filtered

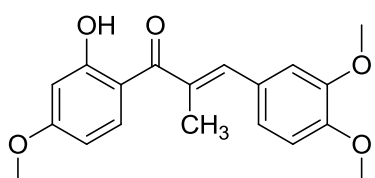
and the remaining solid washed with hot acetone (~30 mL). After evaporating the solvent *in vacuo*, H₂O (35 mL) was added and the resulting mixture was extracted with EtOAc (5 × 30 mL). The combined organic layers were dried over MgSO₄ and the solvent evaporated to afford the pure acetophenone **106** (1.07 g, 5.94 mmol, 99%) as a white solid.

¹H NMR (300 MHz, CDCl₃): δ = 12.84 (s, 1 H; 2'-OH), 7.65 (dd, J = 7.5, 1.8 Hz, 1 H), 6.45-6.42 (m, 2 H), 3.83 (s, 3 H; OCH₃), 2.95 (q, J = 7.3 Hz, 2 H; CH₂), 1.22 (t, J = 7.3 Hz, 3 H; CH₃) ppm.

¹³C NMR (75 MHz, CDCl₃): δ = 205.4 (C=O), 165.9, 165.3, 131.4 (CH), 113.3, 107.5 (CH), 100.9 (CH), 55.6 (OCH₃), 31.2 (CH₂), 8.5 (CH₃) ppm.

The ¹H NMR spectral data is in accordance with the literature.¹¹⁵

(E)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)-2-methylprop-2-en-1-one



(α -Me-HC, **96**).⁵³ A solution of propiophenone **106** (300 mg, 1.66 mmol, 1.0 eq.), 3,4-dimethoxybenzaldehyde (**29**) (276 mg, 1.66 mmol, 1.0 eq.), piperidine (328 μ L, 3.32 mmol, 2 eq.) and glacial AcOH (161 mL, 2.82 mmol, 1.7 eq.) in dry

benzene (8 mL) was heated to reflux using a Dean Stark apparatus for 24 h. The reaction mixture was cooled to room temperature and diluted with EtOAc (60 mL). This mixture was washed with 1 M aq. HCl (15 mL), 1 M aq. NaOH (15 mL), 1 M aq. HCl (15 mL) and then brine (2 × 15 mL). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo* then the product was purified on preparative TLC plates (SiO₂, petroleum ether/EtOAc, 7:3) to afford α -Me-HC (**96**) (300 mg, 914 μ mol, 55%) as a yellow solid; m.p. 82 °C.

¹H NMR (400 MHz, CDCl₃): δ = 12.58 (s, 1 H; 2'-OH), 7.69 (d, J = 8.9 Hz, 1 H), 7.04 (dd, J = 8.3, 1.9 Hz, 1 H), 6.94-6.90 (m, 2 H), 6.84 (d, J = 1.1 Hz, 1 H; β -H), 6.48 (d, J = 2.5 Hz, 1 H), 6.42 (dd, J = 8.9, 2.5 Hz, 1 H), 3.91 (s, 3 H; OCH₃), 3.89 (s, 3 H; OCH₃), 3.84 (s, 3 H; OCH₃), 2.26 (d, J = 1.4 Hz, 3 H; CH₃) ppm.

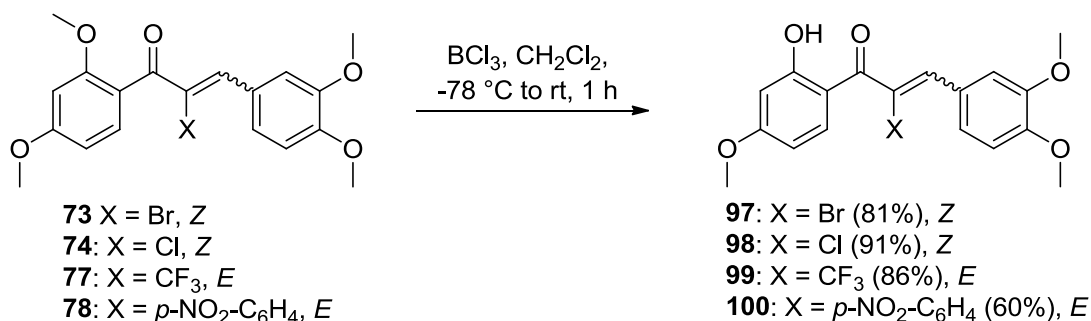
^{13}C NMR (101 MHz, CDCl_3): δ = 202.6 (C=O), 166.1, 165.9, 149.2, 148.7, 137.4 (β -CH), 134.4(CH), 133.9, 128.5, 122.8 (CH), 112.7, 112.6 (CH), 110.9 (CH), 106.9 (CH), 101.1 (CH), 55.9 (OCH_3), 55.9 (OCH_3), 55.5 (OCH_3), 15.6 (CH_3) ppm.

IR (neat): 2937, 2837, 1616, 1579, 1511, 1440, 1338, 1240, 1109, 1023 cm^{-1} .

MS (EI): m/z (%): 329 $[\text{M}+\text{H}]^+$ (100).

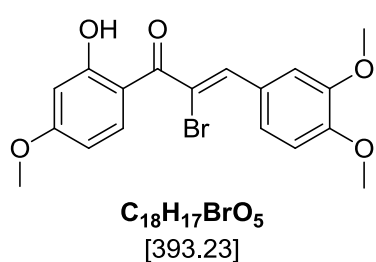
HRMS (EI): calcd. for $\text{C}_{19}\text{H}_{21}\text{O}_5$: 329.1384 $[\text{M}+\text{H}]^+$; found 329.1391.

6.2.12. General Procedure for the synthesis of α -Br/Cl/ CF_3 / p - NO_2 - C_6H_4 -HCs (20-23)



To a solution of α -X-TMCs **73**, **74**, **77** or **78** (1.0 eq.) in dry CH_2Cl_2 was added at -78°C dropwise a 1 M hexane or CH_2Cl_2 solution of BCl_3 (3.0 eq.). Upon addition the yellow solution turned immediately deep-red. After 30 min at -78°C the solution was allowed to warm to 0°C and the solution was stirred for about 1 h. Then, 1.0 mL EtOH and 40 mL H_2O were added to the reaction mixture and the resulting mixture was extracted with CH_2Cl_2 (3 \times 20 mL). The combined organic layers were dried over MgSO_4 , filtered and the solvent removed under reduced pressure. Purification on preparative TLC plates afforded the 2'-OH chalcones **97-100**, respectively.

(Z)-2-Bromo-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one



(α -Br-HC, **97**). The α -bromo-chalcone **97** was prepared according to the general procedure (5.2.12) starting with a solution of α -Br-TMC (**73**) (50.0 mg, 0.123 mmol, 1.0 eq.) in dry CH_2Cl_2 (2.5 mL) and a 1 M solution of BCl_3 in hexane (0.368 mL, 0.368 mmol, 3.0 eq.). Purification on preparative

TLC plates (SiO₂, EtOAc/petroleum ether 3:1) afforded α -Br-HC (**97**) (39.0 mg, 0.0992 mmol, 81 %) as a yellow solid; m.p. 80 °C.

¹H NMR (400 MHz, CDCl₃): δ = 12.06 (s, 1 H, 2'-OH), 7.71 (d, J = 9.0 Hz, 1 H), 7.59 (d, J = 2.0 Hz, 1 H), 7.37 (dd, J = 8.6, 2.0 Hz, 1 H), 7.33 (s, 1 H; β -CH), 6.92 (d, J = 8.4 Hz, 1 H), 6.50 (d, J = 2.5 Hz, 1 H), 6.46 (dd, J = 9.0, 2.5 Hz, 1 H), 3.93 (s, 3 H; OCH₃), 3.93 (s, 3 H; OCH₃), 3.87 (s, 3 H, OCH₃) ppm.

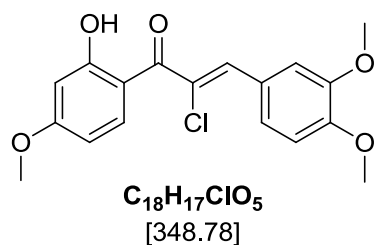
¹³C NMR (101 MHz, CDCl₃): δ = 194.3 (C=O), 166.7, 166.4, 150.6, 148.6, 137.9 (β -CH), 134.3 (CH), 126.1, 124.4 (CH), 115.5, 112.2 (CH), 111.5, 110.7 (CH), 107.8 (CH), 101.2 (CH), 55.9 (2 OCH₃), 55.7 (OCH₃) ppm.

IR (neat): 3004, 2934, 2837, 1624, 1586, 1509, 1440, 1235, 1203, 1127, 1020, 959, 799 cm⁻¹.

MS (ESI⁺) m/z (%): 393 [M+H]⁺ (100).

HRMS (ES-MS): calcd. for C₁₈H₁₈BrO₅: 393.0332 [M+H]⁺; found 393.0336.

(Z)-2-Chloro-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one



(α -Cl-HC, **98**). The α -chloroalcone **98** was prepared according to the general procedure (5.2.12) starting with a solution of α -Cl-TMC (**74**) (50.0 mg, 0.138 mmol, 1.0 eq.) in dry CH₂Cl₂ (2.5 mL) and 1 M solution of BCl₃ in hexane (0.413 mL, 0.413 mmol, 3.0 eq.). Purification on preparative

TLC plates (SiO₂, EtOAc/petroleum ether 3:1) afforded α -Cl-HC (**98**) (44.0 mg, 0.126 mmol, 91%) as a yellow solid; m.p. 89 °C.

¹H NMR (300 MHz, CDCl₃): δ = 12.00 (s, 1 H; 2'-OH), 7.73 (d, J = 8.9 Hz, 1 H), 7.53 (d, J = 2.0 Hz, 1 H), 7.37 (dd, J = 8.5, 2.0 Hz, 1 H), 7.14 (s, 1 H; β -CH), 6.92 (d, J = 8.5 Hz, 1 H), 6.50 (d, J = 2.4 Hz, 1 H), 6.46 (dd, J = 8.9, 2.5 Hz, 1 H), 3.93 (s, 3 H; OCH₃), 3.92 (s, 3 H; OCH₃), 3.86 (s, 3 H; OCH₃) ppm.

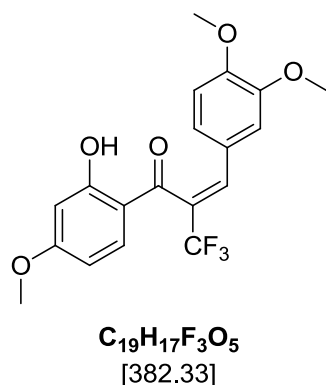
¹³C NMR (101 MHz, CDCl₃): δ = 193.5 (C=O), 166.6, 166.2, 150.6, 148.7, 135.8 (β -CH), 134.0 (CH), 125.6, 125.0, 124.8 (CH), 112.6 (CH), 111.8, 110.8 (CH), 107.7 (CH), 101.2 (CH), 55.9 (2 OCH₃), 55.7 (OCH₃) ppm.

IR (neat): 3005, 2935, 2838, 1620, 1586, 1509, 1440, 1336, 1236, 1128, 1067, 1020, 959, 831 cm^{-1} .

MS (ESI^+) m/z (%): 349 $[\text{M}+\text{H}]^+$ (100).

HRMS (ES-MS): calcd. for $\text{C}_{18}\text{H}_{18}\text{ClO}_5$: 349.0837 $[\text{M}+\text{H}]^+$; found 349.0835.

(Z)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)-2-(trifluoromethyl)prop-



2-en-1-one ($\alpha\text{-CF}_3\text{-HC}$, **99).** The α -trifluoromethylchalcone **99** was prepared according to the general procedure (5.2.12) starting with a solution of $\alpha\text{-CF}_3\text{-TMC}$ (**77**) (46.0 mg, 0.116 mmol, 1.0 eq.) in dry CH_2Cl_2 (2 mL) and a 1 M solution of BCl_3 in CH_2Cl_2 (0.348 mL, 0.348 mmol, 3.0 eq.). Purification by preparative TLC plates (SiO_2 , EtOAc/petroleum ether 2:1) afforded $\alpha\text{-CF}_3\text{-HC}$ (**99**) (38.0 mg, 0.0994 mmol, 86 %) as a pale yellow solid; m.p. 97 $^\circ\text{C}$.

^1H NMR (400 MHz, CDCl_3): δ = 12.34 (s, 1 H; 2'-OH), 7.42 (d, J = 9.0 Hz, 1 H), 7.32 (d, J = 1.5 Hz, 1 H, $\beta\text{-H}$), 6.92 (dd, J = 8.3, 2.1 Hz, 1 H), 6.77-6.73 (m, 2 H), 6.42 (d, J = 2.4 Hz, 1 H), 6.29 (dd, J = 9.0, 2.5 Hz, 1 H), 3.83 (s, 3 H; OCH_3), 3.81 (s, 3 H; OCH_3), 3.64 (s, 3 H; OCH_3) ppm.

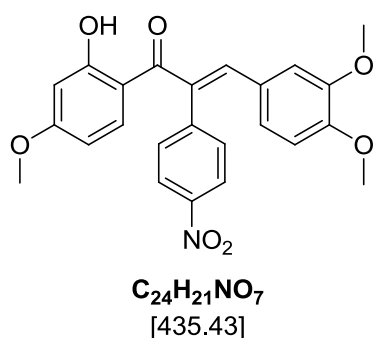
^{13}C NMR (101 MHz, CDCl_3): δ = 195.9 (C=O), 167.4, 166.2, 150.8, 148.8, 136.2 (q, J = 5.8 Hz, $\beta\text{-CH}$), 134.1 (CH), 124.7 (q, J = 30.5 Hz, $\alpha\text{-C}$), 124.5, 124.2 (CH), 122.4 (q, J = 274.1 Hz, CF_3), 113.6, 111.4 (CH), 110.8 (CH), 108.7 (CH), 100.8 (CH), 55.8 (OCH_3), 55.7 (OCH_3), 55.5 (OCH_3) ppm.

^{19}F NMR (376 MHz, CDCl_3): δ = -62.4 (d, J = 1 Hz) ppm.

IR (neat): 3013, 2937, 2842, 1622, 1596, 1515, 1442, 1393, 1238, 1144, 1109, 1023, 833, 616 cm^{-1} .

MS (ESI^+): m/z (%): 383 $[\text{M}+\text{H}]^+$ (100).

HRMS (ES-MS): calcd for $\text{C}_{19}\text{H}_{18}\text{F}_3\text{O}_5$: 383.1101 $[\text{M}+\text{H}]^+$; found 383.1098.

(E)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)-2-(4-nitrophenyl)prop-2-

en-1-one (α -*p*-NO₂-C₆H₄-HC, **100).** The α -aryl-HC **100** was prepared according to the general procedure (5.2.12) starting with a solution of α -*p*-NO₂-C₆H₄-TMC (**78**) (60.0 mg, 0.133 mmol, 1.0 eq) in dry CH₂Cl₂ (3 mL) and a 1 M solution of BCl₃ in CH₂Cl₂ (0.40 mL, 0.399 mmol, 3.0 eq). Purification by preparative TLC plates (SiO₂, EtOAc/petroleum ether 2:1) afforded α -*p*-NO₂-C₆H₄-HC (**100**) (35.0 mg, 0.0804 mmol, 60

%) as a yellow solid; m.p. 68 °C.

¹H NMR (400 MHz, CDCl₃): δ = 12.41 (s, 1 H; 2'-OH), 8.30-8.15 (m, 2 H), 7.73 (d, *J* = 9.0 Hz, 1 H), 7.60-7.52 (m, 2 H), 7.08 (s, 1 H; β -H), 6.74 (d, *J* = 0.8 Hz, 2 H), 6.57-6.48 (m, 2 H), 6.44 (dd, *J* = 9.0, 2.5 Hz, 1 H), 3.87 (s, 6 H; 2 OCH₃), 3.55 (s, 3 H; OCH₃) ppm.

¹³C NMR (101 MHz, CDCl₃): δ = 199.1 (C=O), 166.6, 166.5, 150.3, 148.6, 147.3, 144.1, 138.7 (β -CH), 135.4, 134.3 (CH), 130.9 (2 CH), 126.3, 124.1 (CH), 124.0 (2 CH), 112.9, 112.5 (CH), 110.9 (CH), 107.7 (CH), 101.3 (CH), 55.9 (OCH₃), 55.7 (OCH₃), 55.4 (OCH₃) ppm.

IR (neat): 2934, 2839, 1677, 1604, 1514, 1442, 1343, 1254, 1159, 1021, 808 cm⁻¹.

MS (ESI⁺): *m/z* (%): 436 [M+H]⁺ (100).

HRMS (ES-MS): calcd for C₂₄H₂₂NO₇: 436.1391 [M+H]⁺; found 436.1391.

6.3. Kinetic thiol assay

6.3.1. Standard assay (96-well-format) for compounds with middle to low reactivity

The thiol assay was conducted in 96-well plates (ELISA Microplates, F-bottom, MICROLON 200, Greiner Bio-one, Germany) and PCR foils (Viewseal transparent, 80×140 mm, Greiner Bio-one, USA) were applied to cover the plate during the measurements. As a solvent system 100 mM TRIS-HCl buffer pH 7.4 with 2 mM EDTA/ethylene glycol (20:80) was used that was filtered and degassed prior to use. All measurements were done in a Thermo Scientific Multiskan Spectrum UV/Vis microplate and cuvette spectrophotometer (Thermo Fisher Scientific Inc., Finland) at 25 °C. To perform the assay 10 mM stock solutions of the chalcone in DMSO were diluted in the buffer/ethylene glycol mixture to give a concentration of 80 μ M

(160 μM for chalcone **71** and **77**). Stock solutions with a concentration of 40 mM of the respective thiol (cysteamine-HCl (**107**), 1,4-butanedithiol (**109**), 2-mercaptoethanol (**111**) or glutathione (GSH, **112**)) were directly prepared in the buffer/ethylene glycol mixture and diluted accordingly to give 12-500 fold dilutions compared to the α,β -unsaturated carbonyl compounds. Suitable wavelengths for the individual compounds were determined by photoscan experiments ($\lambda = 290\text{-}600\text{ nm}$) of the tested chalcones with the thiol together with LC-MS measurements to rule out an overlap of UV bands of the starting material with the products. Chalcone and thiol solutions were incubated separately in the instrument for 10 min at 25 $^{\circ}\text{C}$ just prior to the measurement. Then, equal amounts of both solutions were combined, mixed thoroughly, the wells were covered with foil, and the kinetic measurement was started immediately to be as close as possible to the starting concentration of 40 μM (80 μM in case of chalcone **71** and **77**) for the chalcones. Figure 53 shows the detailed protocol of this assay.

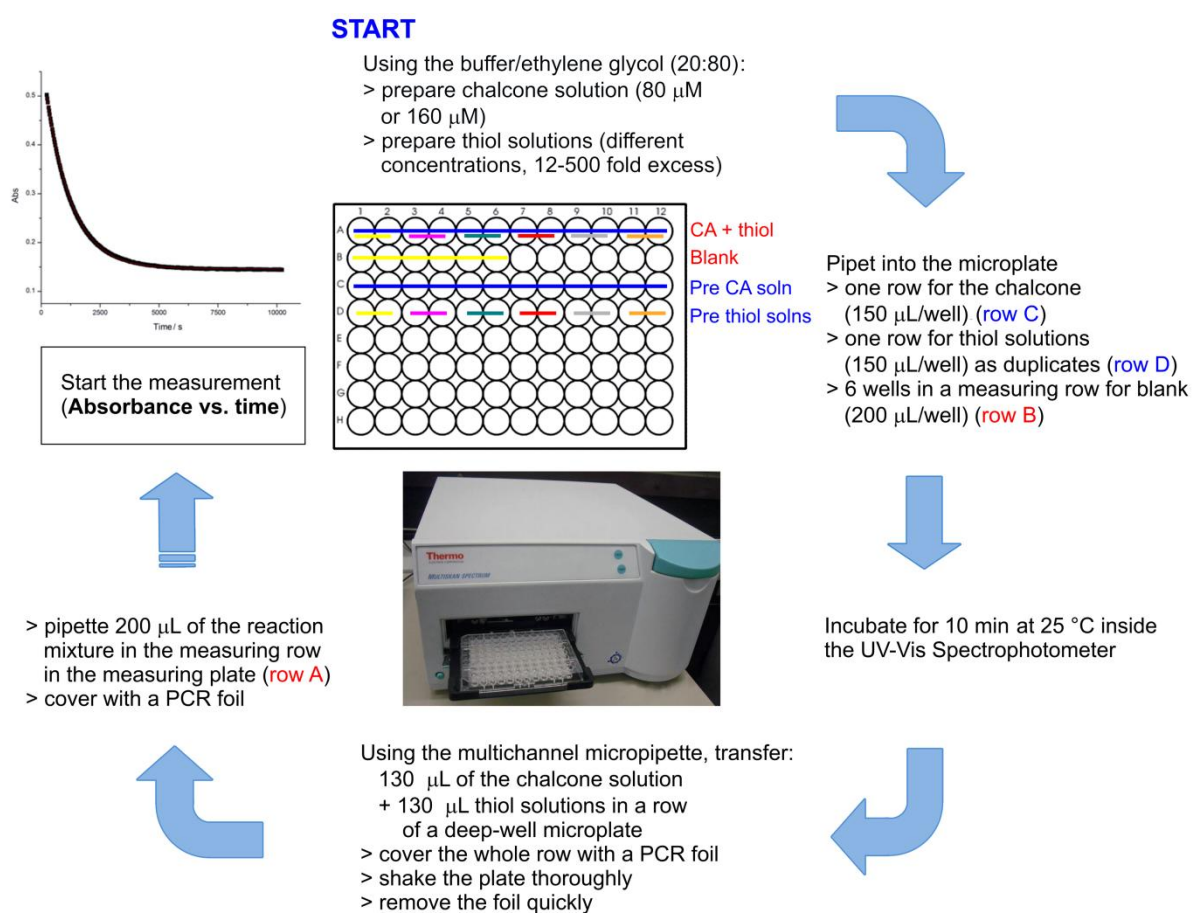


Figure 53: The kinetic thiol assay protocol.

Measurements were done in duplicates. A set of 380 data points (absorbances A_t) was collected each time with varying time intervals (Δt). The raw data were corrected versus the

blank using an Excel data sheet that was programmed by Karl Amslinger to give individual curves for each measurement. These curves are fit to a first-order exponential decay function with the software OriginPro 8 (OriginLab Corporation, USA) to gain individual k_{obs} values. The k_2 values were calculated on an Excel data sheet by linear regression of the obtained k_{obs} values versus their corresponding thiol concentrations. All compounds were measured with 3-7 independent experiments.

6.3.2. Stopped-flow assay for compounds with high reactivity

The stopped-flow measurements were performed at 25 °C in the same solvent system utilized in the microtiter plate assay which is 100 mM TRIS-HCl buffer pH 7.4 with 2 mM EDTA/ethylene glycol (20:80), by using the SX20 stopped-flow instrument (Applied Photophysics, UK). The decrease in the absorbance at the suitable wavelength of each chalcone was recorded over time. Multiple turnover kinetics of a certain chalcone with a thiol were measured by mixing 80 μM solutions of chalcones (160 μM solutions of chalcone **71** and **77**) in buffer/ethylene glycol (20:80) and the corresponding solutions of thiol (in buffer/ethylene glycol (20:80), different concentrations, 12-500-fold) in a 1:1 volume ratio. The final concentration of chalcones was 40 μM (80 μM for chalcone **71** and **77**). Three to five traces were recorded at each thiol concentration and averaged. These curves were fit to a first-order exponential decay function by using the software Pro-Data SX 2.0 (Applied Photophysics, UK) and the k_2 values were calculated as in the standard assay. Three independent experiments were performed for each chalcone

6.4. LC-MS study of the thiol assay samples

Mass spectrometric investigations were carried out on an Agilent 1290 Infinity HPLC system coupled to Agilent Technologies 6540 UHD TOF/Q-TOF mass spectrometer (USA). For chromatographic separation, Agilent Zorbax Eclipse Plus C18, 1.8 μm , 50 \times 2.1 mm column was used at 40 °C and detection wavelength range was 190-640 nm. Using 0.1% formic acid/water (eluent A) and 0.1% formic acid/MeCN (eluent B), the following gradient at a constant flow rate of 0.60 mL/min was applied: 0-4 min, 95% A, 5% B; 4-5.1 min, 2% A, 98% B; 5.1-6 min, 95% A, 5% B. The following parameters for MS data acquisition were applied: operation mode: positive; capillary voltage: 3500 V; dry gas flow (N_2): 8 L/min; nebulizer pressure: 35 psi and capillary temperature: 320 °C. Mass spectra were recorded between m/z 80 and 1400. The software used to control the LC-MS system and process data was a MassHunter

Workstation (Agilent, USA). Compounds were identified according to their HR-MS, specific fragmentation pattern, UV spectra and retention times.

6.5. Biological tests

Tests were performed on murine macrophages (RAW264.7 cells) that were cultured in RPMI 1640 medium supplemented with 10% (v/v) heat inactivated fetal calf serum (FCS) and 2 mM glutamine (Biochrom AG, Germany) at 37 °C in humidified air containing 5% CO₂.

6.5.1. In vitro cell viability assay

The cell viability was evaluated by MTT assay. In brief, RAW264.7 cells were seeded in 96-well plates at a density of 5×10^3 per well, cultured for 24 h, and then incubated for 20 h with medium supplemented with compounds (for compound preparation see iNOS inhibition test below) in presence and absence of 10 ng mL⁻¹ LPS (Sigma, Germany). Controls received only culture medium with or without solvent, both in presence and absence of LPS. The total assay volume was 100 μ L and the final concentration of the solvent (DMSO) was $\leq 0.025\%$. After the 20 h of incubation, 10 μ L of MTT solution (4 mg mL⁻¹ in phosphate-buffered saline (PBS)) was added to each well, and the cells were incubated for another 4 h at 37 °C. Subsequently, the culture medium was removed from the wells, then 100 μ L of sodium dodecyl sulfate (SDS) solution (10% in water) was added, and the formed formazan dye was allowed to dissolve overnight. The absorbance was determined at $\lambda = 560$ nm with a Multiskan Spectrum spectrophotometer (Thermo Fisher Scientific Inc., Finland) at 25 °C. Figure 54 represents a summary of this protocol. The data represent the mean standard deviation (\pm SD) of at least three independent experiments carried out in quadruplicates.

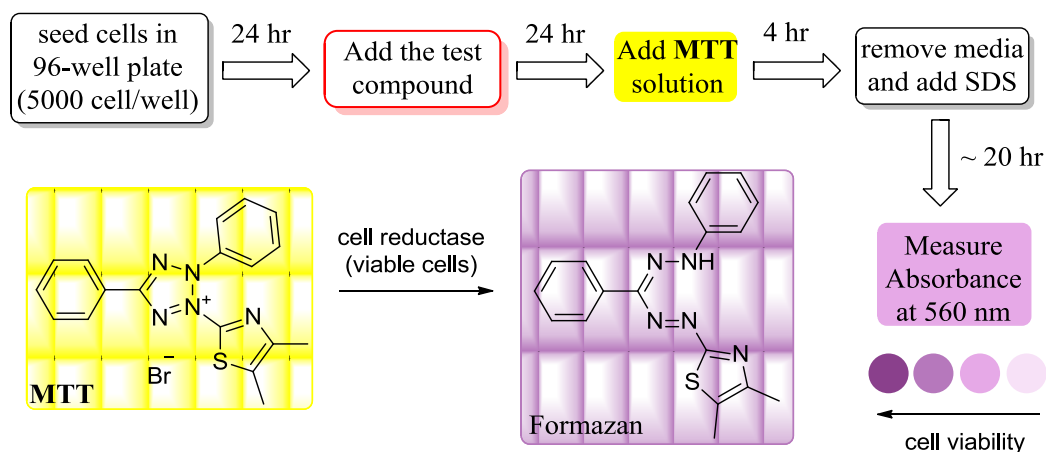


Figure 54: MTT assay protocol with relevant reaction of the yellow MTT to give the purple formazan dye.

6.5.2. iNOS inhibition test (Griess assay)

The produced NO, which is accumulated as nitrite in the culture medium, was quantified using the Griess reaction. RAW264.7 macrophages (8×10^4 cells per well) were plated in 96-well plates and allowed to attach for 24 h. Stock solutions of the compounds were prepared in DMSO (100 mM) and stored at $-20\text{ }^{\circ}\text{C}$. Test concentrations were freshly prepared by diluting the stock solution in culture medium in presence and absence of 10 ng mL^{-1} LPS and then incubated with the cells for 24 h. The final concentration of DMSO was $\leq 0.025\%$ and the total assay volume was $100\text{ }\mu\text{L}$. An amount of $50\text{ }\mu\text{L}$ of the culture medium was mixed with $50\text{ }\mu\text{L}$ of the Griess reagent (0.1% NED (*N*-1-naphtylethylenediamine dihydrochloride), 1% sulfanilamide, 0.35% phosphoric acid in water) and incubated for 15 min at room temperature. During this time the formed air bubbles upon pipetting are removed using nitrogen stream. The absorbance was measured at $\lambda = 560\text{ nm}$ with a Multiskan Spectrum Photometer (Thermo Fisher Scientific Inc., Finland) at $25\text{ }^{\circ}\text{C}$. The assay protocol is depicted in Figure 55. The data represent the mean \pm SD of at least three independent experiments carried out in quadruplicates.

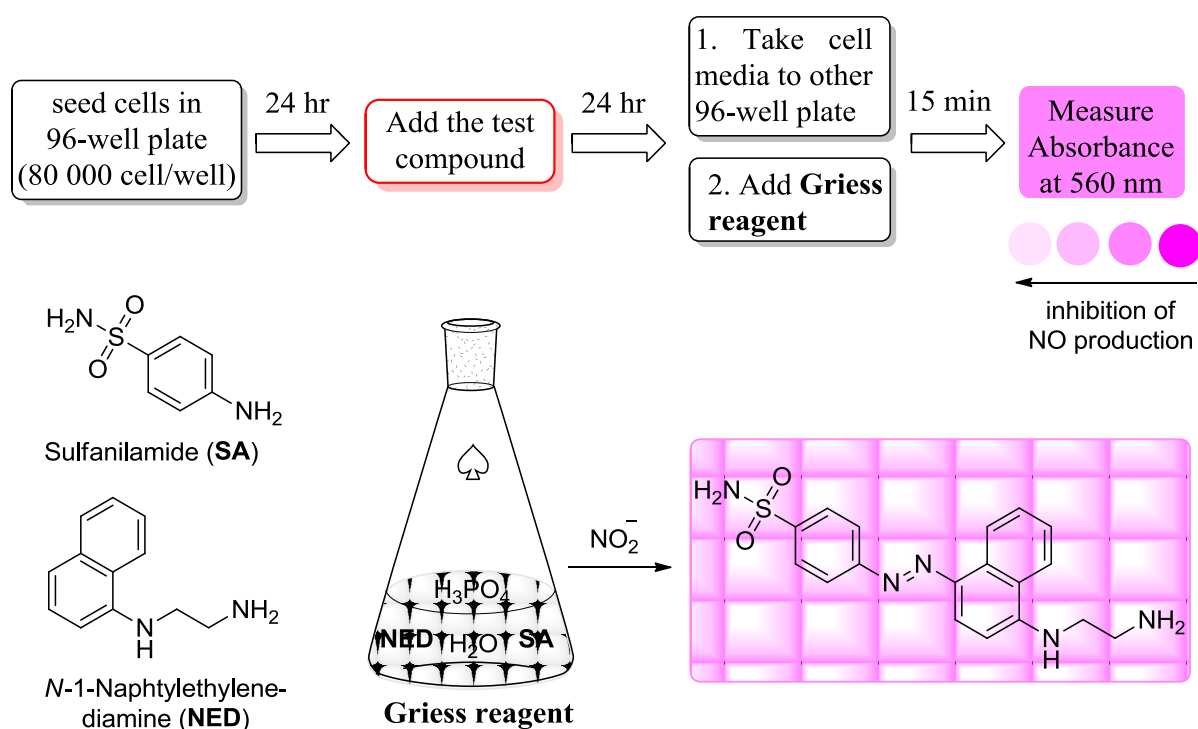
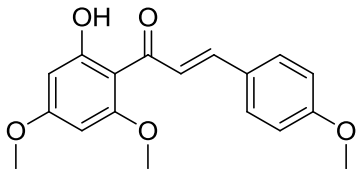
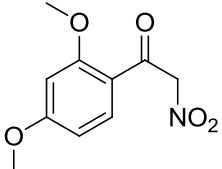
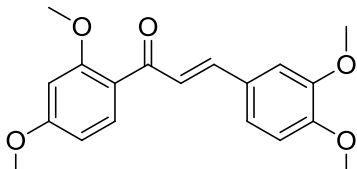
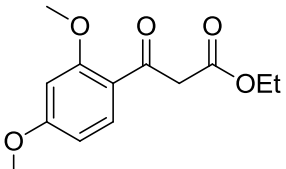
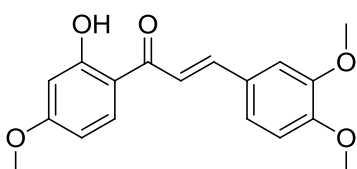
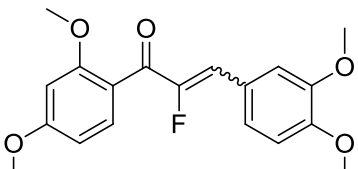
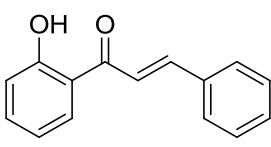
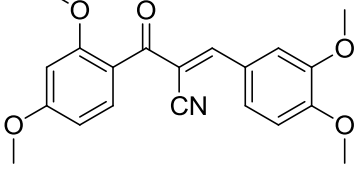
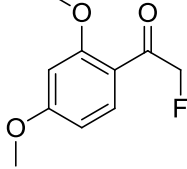
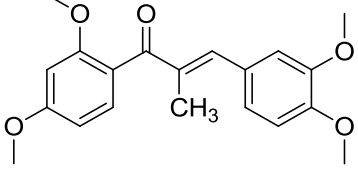
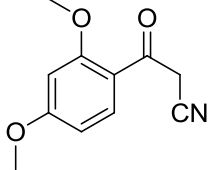
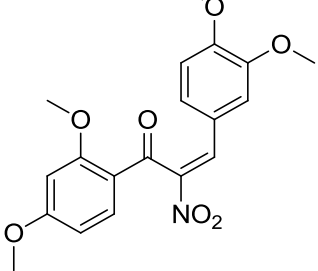
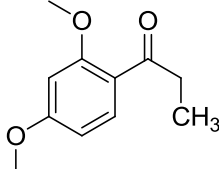
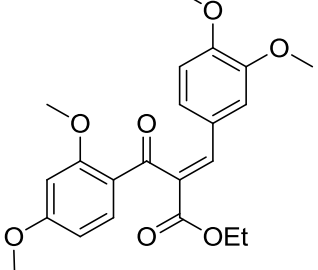


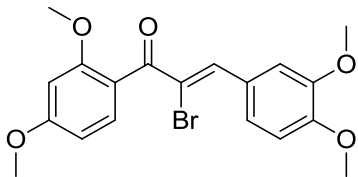
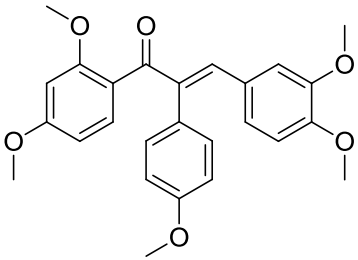
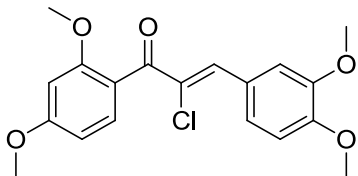
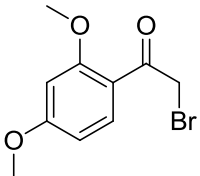
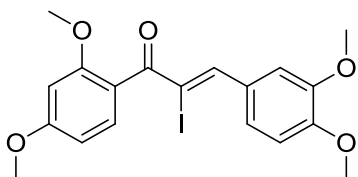
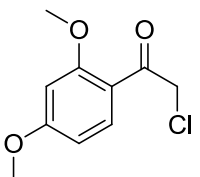
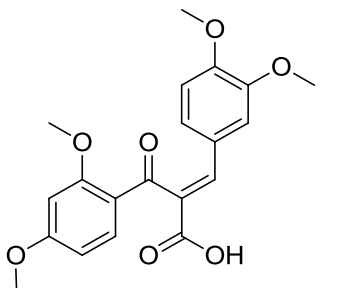
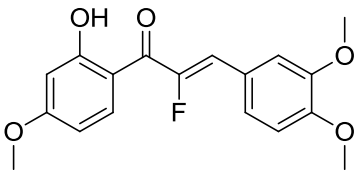
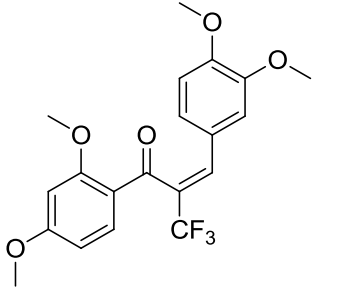
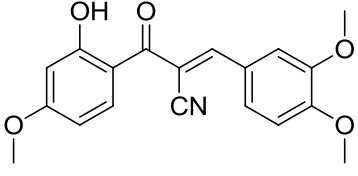
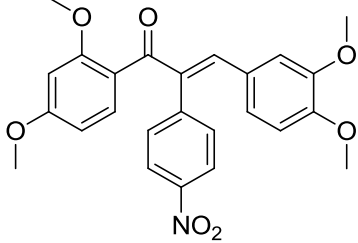
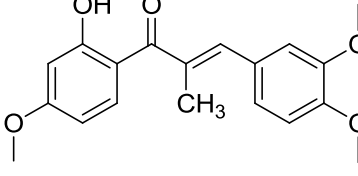
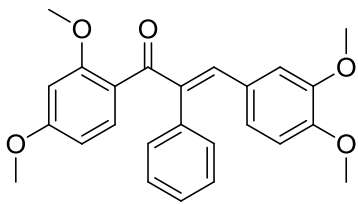
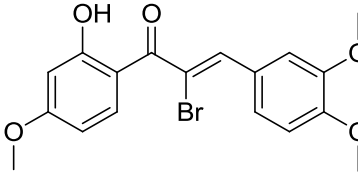
Figure 55: The Griess assay protocol.

7. APPENDIX

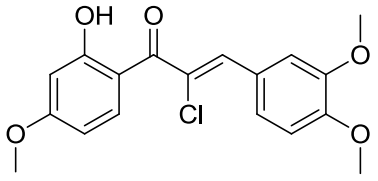
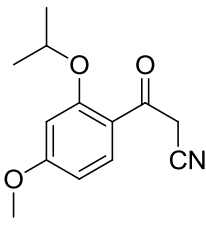
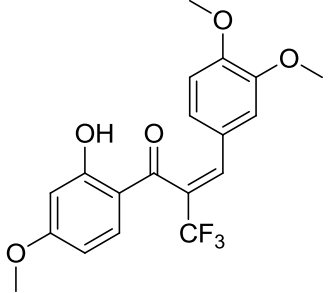
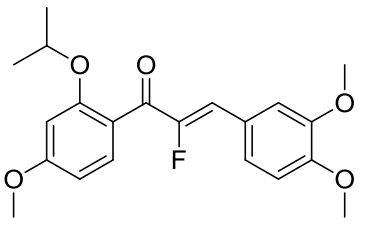
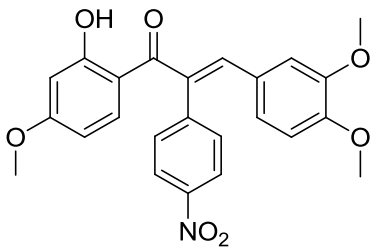
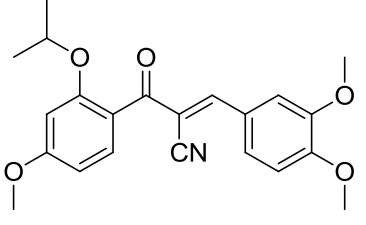
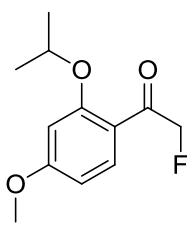
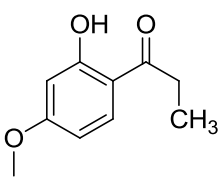
7.1. Table of the synthesized compounds

#	Assigned structure	#	Assigned structure
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60		67	
61		68	
62		69	
63		70	
64		71	
65		72	

APPENDIX

#	Assigned structure	#	Assigned structure
73		80	
74		81	
75		82	
76		94	
77		95	
78		96	
79		97	

APPENDIX

#	Assigned structure	#	Assigned structure
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99		104	
100		105	
102		106	

7.2.X-Ray data

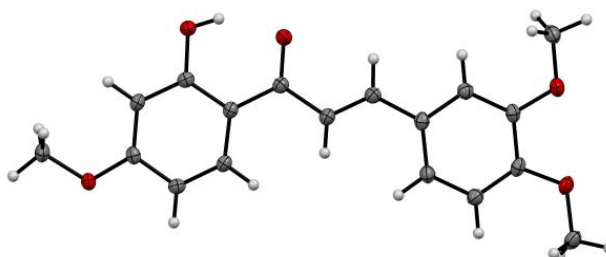
7.2.1. (E)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxy-phenyl)prop-2-en-1-one (α -H-HC, 61)

CCDC no.: 909409.

The dihedral angle between the two aromatic rings is: 11.38°.

Crystal data

$C_{18}H_{18}O_5$	$zV = 1499.16 (9) \text{ \AA}^3$
$M_r = 314.32$	$Z = 4$
Monoclinic, $P2_1/c$	Cu $K\alpha$ radiation
$a = 17.9253 (7) \text{ \AA}$	$\mu = 0.84 \text{ mm}^{-1}$
$b = 5.36599 (19) \text{ \AA}$	$T = 123 \text{ K}$
$c = 15.7715 (5) \text{ \AA}$	$0.29 \times 0.14 \times 0.04 \text{ mm}$
$\beta = 98.798 (3)^\circ$	



Data collection

SuperNova, Single source at offset), Atlas diffractometer	2974 independent reflections
Absorption correction: analytical <i>CrysAlis PRO</i> , Oxford Diffraction Ltd., Version 1.171.33.55 (release 05-01-2010 <i>CrysAlis171 .NET</i>) Analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by R.C. Clark & J.S. Reid. (Clark, R. C. & Reid, J. S. (1995). <i>Acta Cryst.</i> A51, 887-897)	2642 reflections with $I > 2\sigma(I)$
$T_{\min} = 0.832$, $T_{\max} = 0.965$	$R_{\text{int}} = 0.024$
11609 measured reflections	$\theta_{\max} = 73.6^\circ$

Refinement

$R[F^2 > 2\sigma(F^2)] = 0.036$	0 restraints
$wR(F^2) = 0.096$	H-atom parameters constrained
$S = 1.03$	$\Delta\rho_{\max} = 0.20 \text{ e \AA}^{-3}$
2974 reflections	$\Delta\rho_{\min} = -0.29 \text{ e \AA}^{-3}$
208 parameters	

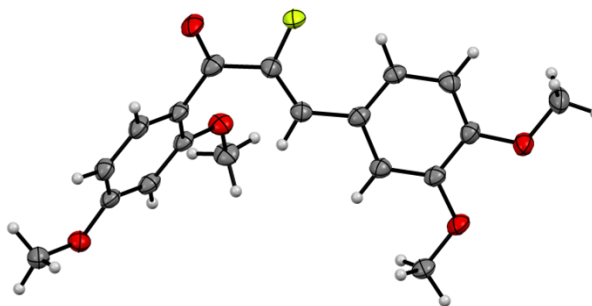
7.2.2. (Z)-2-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-fluoroprop-2-en-1-one (α -F-TMC, 68)

CCDC no.: 9111005.

The dihedral angle between the two aromatic rings is: 54.33°.

Crystal data

$C_{19}H_{19}FO_5$	$V = 810.16 (10) \text{ \AA}^3$
$M_r = 346.34$	$Z = 2$
Monoclinic, $P2_1$	Cu $K\alpha$ radiation
$a = 4.1742 (3) \text{ \AA}$	$\mu = 0.92 \text{ mm}^{-1}$
$b = 16.3559 (12) \text{ \AA}$	$T = 123 \text{ K}$
$c = 11.9009 (7) \text{ \AA}$	$0.18 \times 0.07 \times 0.02 \text{ mm}$
$\beta = 94.353 (6)^\circ$	



Data collection

SuperNova, Single source at offset, Atlas diffractometer	2381 independent reflections
Absorption correction: analytical CrysAlisPro, Oxford Diffraction Ltd., Version 1.171.33.55 (release 05-01-2010 CrysAlis171 .NET) Analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by R.C. Clark & J.S. Reid. (R. C. Clark, J. S. Reid, <i>Acta Crystallogr., Sect. A</i> 1995 , 51, 887-897)	2098 reflections with $I > 2\sigma(I)$
$T_{\min} = 0.907$, $T_{\max} = 0.979$	$R_{\text{int}} = 0.046$
3620 measured reflections	$\theta_{\max} = 72.9^\circ$

Refinement

$R[F^2 > 2\sigma(F^2)] = 0.052$	H-atom parameters constrained
$wR(F^2) = 0.140$	$\Delta\rho_{\max} = 0.23 \text{ e \AA}^{-3}$
$S = 1.04$	$\Delta\rho_{\min} = -0.26 \text{ e \AA}^{-3}$
2381 reflections	Absolute structure: H. Flack, <i>Acta Crystallogr., Sect. A</i> 1983 , 39, 876-881.
226 parameters	Flack parameter: $-0.1 (2)$
1 restraint	

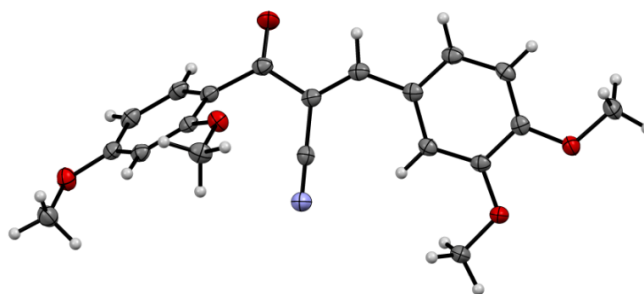
7.2.3. (E)-2-Cyano-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (α -CN-TMC, 69)

CCDC no.: 910997

The dihedral angle between the two aromatic rings is: 73.81°.

Crystal data

$C_{20}H_{19}NO_5$	$\gamma = 80.585 (5)^\circ$
$M_r = 353.36$	$V = 861.54 (9) \text{ \AA}^3$
Triclinic, P	$Z = 2$
$a = 8.7545 (6) \text{ \AA}$	Cu $K\alpha$ radiation
$b = 8.7992 (5) \text{ \AA}$	$\mu = 0.81 \text{ mm}^{-1}$
$c = 11.5752 (7) \text{ \AA}$	$T = 123 \text{ K}$
$\alpha = 86.825 (5)^\circ$	$0.38 \times 0.31 \times 0.10 \text{ mm}$
$\beta = 78.429 (5)^\circ$	



Data collection

SuperNova, Single source at offset), Atlas diffractometer	3192 independent reflections
Absorption correction: analytical CrysAlisPro, Oxford Diffraction Ltd., Version 1.171.33.55 (release 05-01-2010 CrysAlis171 .NET) Analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by R.C. Clark & J.S. Reid. (R. C. Clark, J. S. Reid, <i>Acta Crystallogr., Sect. A</i> 1995 , 51, 887- 897)	2967 reflections with $I > 2\sigma(I)$
$T_{\min} = 0.838$, $T_{\max} = 0.931$	$R_{\text{int}} = 0.012$
6077 measured reflections	$\theta_{\max} = 73.7^\circ$

Refinement

$R[F^2 > 2\sigma(F^2)] = 0.033$	0 restraints
$wR(F^2) = 0.088$	H-atom parameters constrained
$S = 1.06$	$\Delta\rho_{\max} = 0.21 \text{ e \AA}^{-3}$
3192 reflections	$\Delta\rho_{\min} = -0.19 \text{ e \AA}^{-3}$
235 parameters	

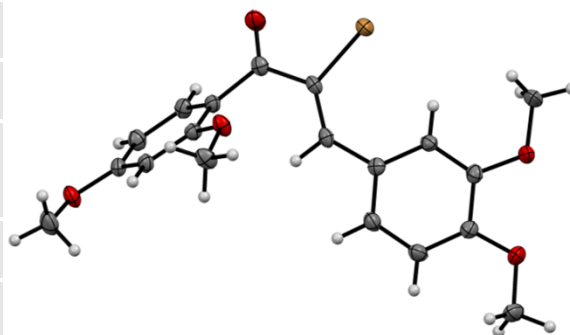
7.2.4. (Z)-2-Bromo-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (α -Br-TMC, 73)

CCDC no.: 910973

The dihedral angle between the two aromatic rings is: 61.37°.

Crystal data

$C_{19}H_{19}BrO_5$	$V = 1791.9 (4) \text{ \AA}^3$
$M_r = 407.24$	$Z = 4$
Orthorhombic, $Pca2_1$	Mo $K\alpha$ radiation
$a = 15.281 (3) \text{ \AA}$	$\mu = 2.32 \text{ mm}^{-1}$
$b = 13.5346 (17) \text{ \AA}$	$T = 123 \text{ K}$
$c = 8.6638 (5) \text{ \AA}$	$0.84 \times 0.08 \times 0.06 \text{ mm}$



Data collection

Xcalibur, Ruby, Gemini Ultra diffractometer	5088 independent reflections
Absorption correction: gaussian CrysAlisPro, Agilent Technologies, Version 1.171.35.21 (release 20-01-2012 CrysAlis171.NET) (compiled Jan 23 2012, 18:06:46) Numerical absorption correction based on gaussian integration over a multifaceted crystal model	4563 reflections with $> 2\sigma(i)$
$T_{\min} = 0.440$, $T_{\max} = 2.163$	$R_{\text{int}} = 0.056$
20133 measured reflections	$\theta_{\max} = 30.8^\circ$

Refinement

$R[F^2 > 2\sigma(F^2)] = 0.041$	H-atom parameters constrained
$wR(F^2) = 0.101$	$\Delta\rho_{\max} = 0.99 \text{ e \AA}^{-3}$
$S = 1.04$	$\Delta\rho_{\min} = -0.68 \text{ e \AA}^{-3}$
5088 reflections	Absolute structure: H. Flack, <i>Acta Crystallogr., Sect. A</i> 1983, 39, 876-881.
230 parameters	Flack parameter: $-0.018 (9)$
1 restraint	

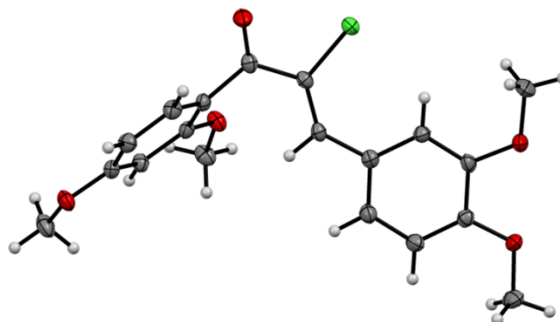
7.2.5. (Z)-2-Chloro-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (α -Cl-TMC, 74)

CCDC no.: 911020

The dihedral angle between the two aromatic rings is: 61.26°.

Crystal data

$C_{19}H_{19}ClO_5$	$V = 1752.73 (4) \text{ \AA}^3$
$M_r = 362.79$	$Z = 4$
Orthorhombic, $Pca2_1$	Cu $K\alpha$ radiation
$a = 15.1477 (2) \text{ \AA}$	$\mu = 2.16 \text{ mm}^{-1}$
$b = 13.4641 (2) \text{ \AA}$	$T = 123 \text{ K}$
$c = 8.5939 (1) \text{ \AA}$	$0.77 \times 0.07 \times 0.04 \text{ mm}$



Data collection

SuperNova, Single source at offset, Atlas diffractometer	2614 independent reflections
Absorption correction: gaussian CrysAlisPro, Agilent Technologies, Version 1.171.35.21 (release 20-01-2012 CrysAlis171 .NET) (compiled Jan 23 2012, 18:06:46) Numerical absorption correction based on gaussian integration over a multifaceted crystal model	2544 reflections with $> 2\sigma(i)$
$T_{\min} = 0.523$, $T_{\max} = 1.382$	$R_{\text{int}} = 0.047$
9295 measured reflections	$\theta_{\max} = 70.8^\circ$

Refinement

$R[F^2 > 2\sigma(F^2)] = 0.030$	H-atom parameters constrained
$wR(F^2) = 0.076$	$\Delta\rho_{\max} = 0.17 \text{ e \AA}^{-3}$
$S = 1.12$	$\Delta\rho_{\min} = -0.23 \text{ e \AA}^{-3}$
2614 reflections	Absolute structure: H. Flack, <i>Acta Crystallogr., Sect. A</i> 1983 , 39, 876-881.
230 parameters	Flack parameter: 0.020 (16)
1 restraint	

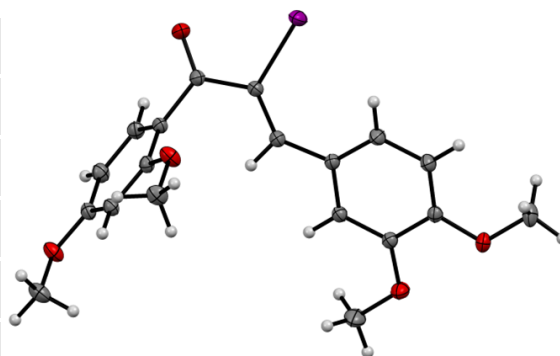
7.2.6. (Z)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-iodoprop-2-en-1-one (α -I-TMC, 75)

CCDC no.: 911012

The dihedral angle between the two aromatic rings is: 78.63°.

Crystal data

$C_{19}H_{19}IO_5$	$V = 1803.12 (7) \text{ \AA}^3$
$M_r = 454.24$	$Z = 4$
Monoclinic, $P2_1/c$	Mo $K\alpha$ radiation
$a = 9.4013 (2) \text{ \AA}$	$\mu = 1.80 \text{ mm}^{-1}$
$b = 23.4507 (4) \text{ \AA}$	$T = 123 \text{ K}$
$c = 8.5471 (2) \text{ \AA}$	$0.34 \times 0.29 \times 0.12 \text{ mm}$
$\beta = 106.885 (2)^\circ$	



Data collection

Xcalibur, Ruby, Gemini Ultra diffractometer	9277 independent reflections
Absorption correction: multi-scan CrysAlisPro, Agilent Technologies, Version 1.171.35.21 (release 20-01-2012 CrysAlis171 .NET) (compiled Jan 23 2012, 18:06:46) Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.	8406 reflections with $> 2\sigma(i)$
$T_{\min} = 0.784$, $T_{\max} = 1.000$	$R_{\text{int}} = 0.025$
47651 measured reflections	$\theta_{\max} = 37.7^\circ$

Refinement

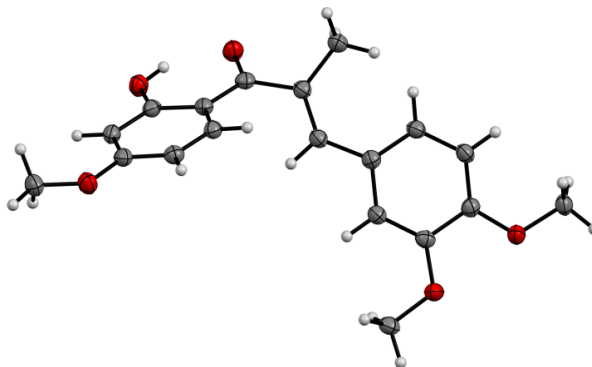
$R[F^2 > 2\sigma(F^2)] = 0.022$	0 restraints
$wR(F^2) = 0.059$	H-atom parameters constrained
$S = 1.06$	$\Delta\rho_{\max} = 0.81 \text{ e \AA}^{-3}$
9277 reflections	$\Delta\rho_{\min} = -1.03 \text{ e \AA}^{-3}$
230 parameters	

7.2.7. (E)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)-2-methylprop-2-en-1-one (α -Me-HC, 96)

The dihedral angle between the two aromatic rings is: 63.61°.

Crystal data

C ₁₉ H ₂₀ O ₅	$\gamma = 82.771 (4)^\circ$
Mr = 328.35	V = 801.83 (7) Å ³
Triclinic, P	Z = 2
a = 5.5747 (3) Å	Cu K α radiation
b = 11.0856 (5) Å	$\mu = 0.81 \text{ mm}^{-1}$
c = 13.7020 (7) Å	T = 123 K
$\alpha = 73.158 (4)^\circ$	0.19 × 0.10 × 0.03 mm
$\beta = 83.900 (4)^\circ$	



Data collection

SuperNova, Single source at offset), Atlas diffractometer	3090 independent reflections
Absorption correction: analytical CrysAlisPro, Oxford Diffraction Ltd., Version 1.171.33.55 (release 05-01-2010 CrysAlis171 .NET) Analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by R.C. Clark & J.S. Reid. (R. C. Clark, J. S. Reid, <i>Acta Crystallogr., Sect. A</i> 1995 , 51, 887-897)	2572 reflections with $I > 2\sigma(I)$
$T_{\min} = 0.911$, $T_{\max} = 0.979$	$R_{\text{int}} = 0.034$
6642 measured reflections	$\theta_{\max} = 73.7^\circ$

Refinement

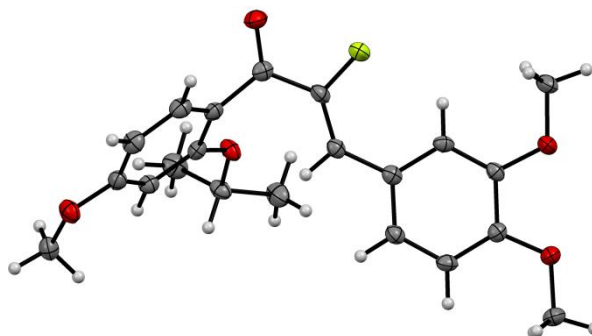
$R[F^2 > 2\sigma(F^2)] = 0.050$	0 restraints
$wR(F^2) = 0.144$	H atoms treated by a mixture of independent and constrained refinement
$S = 1.06$	$\Delta\rho_{\max} = 0.56 \text{ e \AA}^{-3}$
3090 reflections	$\Delta\rho_{\min} = -0.56 \text{ e \AA}^{-3}$
220 parameters	

7.2.8. (Z)-3-(3,4-Dimethoxyphenyl)-2-fluoro-1-(2-isopropoxy-4-methoxyphenyl)prop-2-en-1-one (α -F-pHC, 104)

The dihedral angle between the two aromatic rings is: 64.46°

Crystal data

$C_{21}H_{23}FO_5$	$V = 1882.26 (10) \text{ \AA}^3$
$M_r = 374.39$	$Z = 4$
Monoclinic, $P2_1/c$	Cu $K\alpha$ radiation
$a = 11.8439 (4) \text{ \AA}$	$\mu = 0.83 \text{ mm}^{-1}$
$b = 8.6028 (2) \text{ \AA}$	$T = 123 \text{ K}$
$c = 18.9320 (6) \text{ \AA}$	$0.10 \times 0.08 \times 0.05 \text{ mm}$
$\beta = 102.638 (3)^\circ$	



Data collection

SuperNova, Single source at offset), Atlas diffractometer	2928 independent reflections
Absorption correction: analytical CrysAlis PRO, Oxford Diffraction Ltd., Version 1.171.33.55 (release 05-01-2010 CrysAlis171 .NET) Analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by R.C. Clark & J.S. Reid. (R. C. Clark, J. S. Reid, <i>Acta Crystallogr., Sect. A</i> 1995 , 51, 887-897)	2448 reflections with $I > 2\sigma(I)$
$T_{\min} = 0.956$, $T_{\max} = 0.978$	$R_{\text{int}} = 0.028$
7936 measured reflections	$\theta_{\max} = 63.2^\circ$

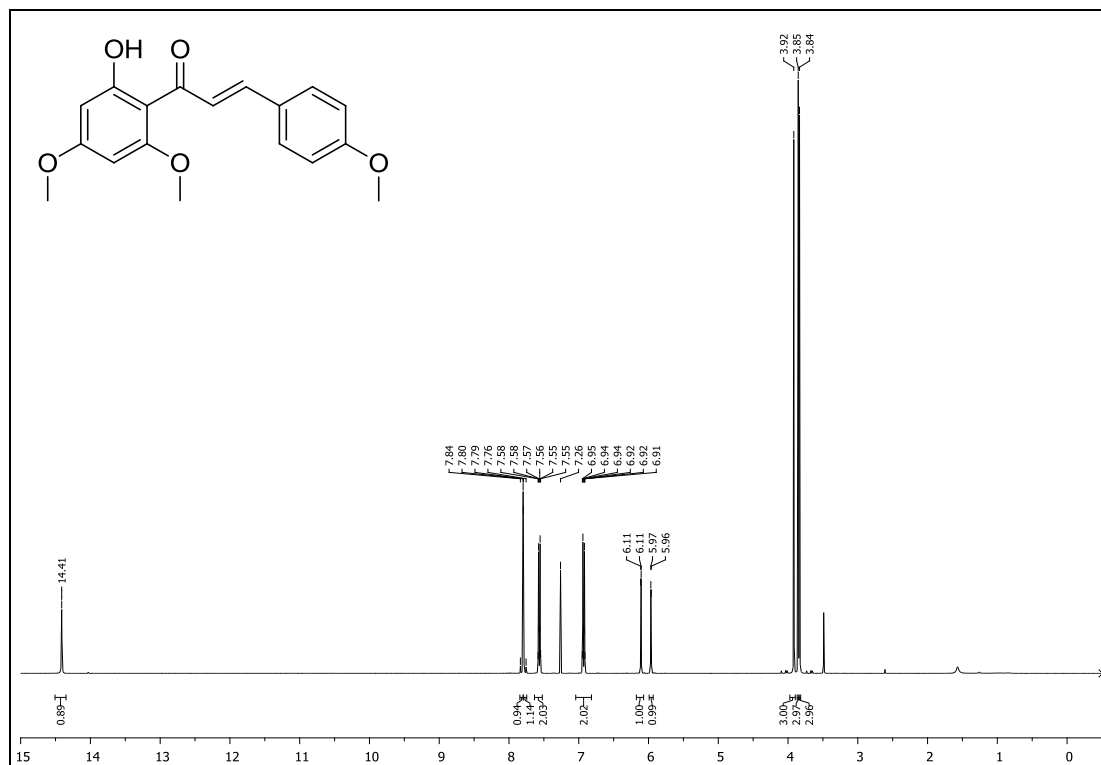
Refinement

$R[F^2 > 2\sigma(F^2)] = 0.036$	0 restraints
$wR(F^2) = 0.091$	H-atom parameters constrained
$S = 1.05$	$\Delta\rho_{\max} = 0.15 \text{ e \AA}^{-3}$
2928 reflections	$\Delta\rho_{\min} = -0.19 \text{ e \AA}^{-3}$
244 parameters	

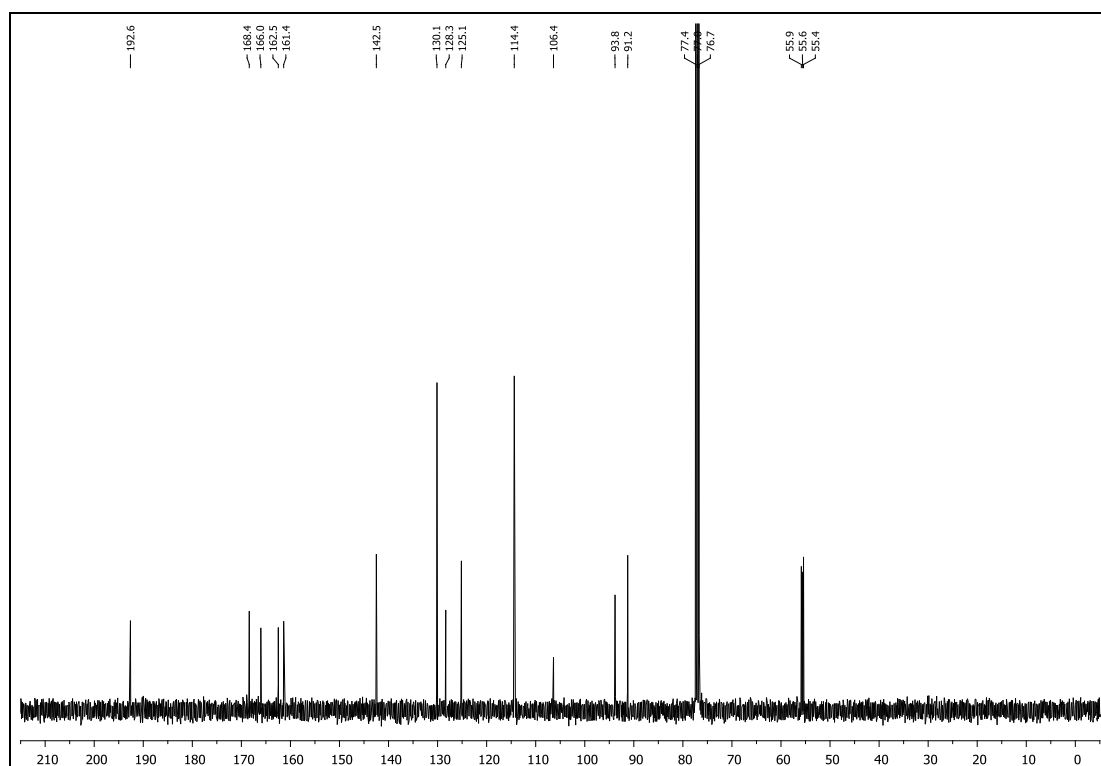
7.3. NMR Spectra

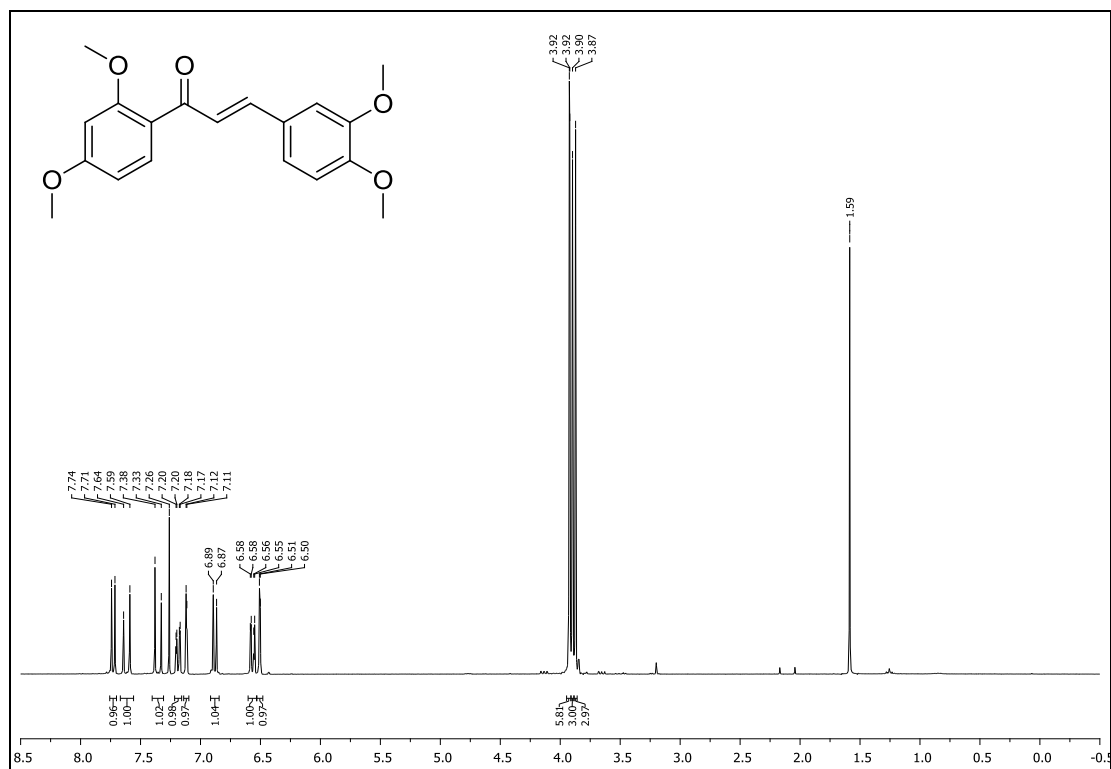
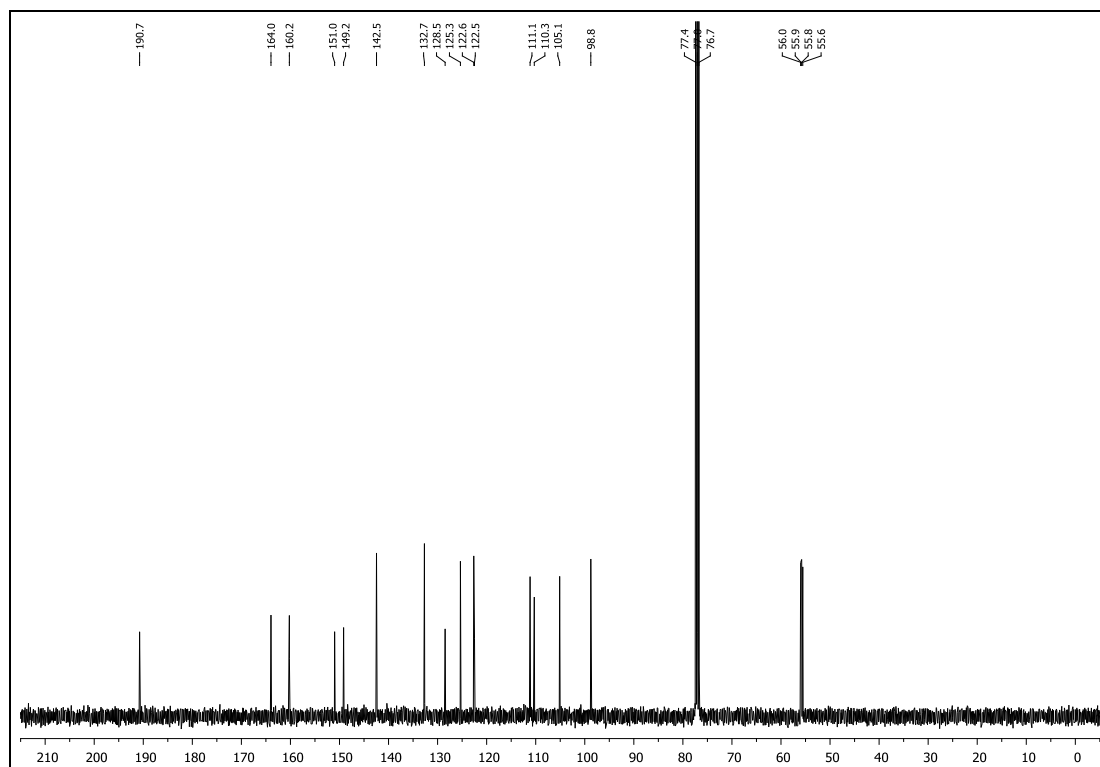
(*E*)-1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **(Flavokawain A) (3)**

¹H NMR



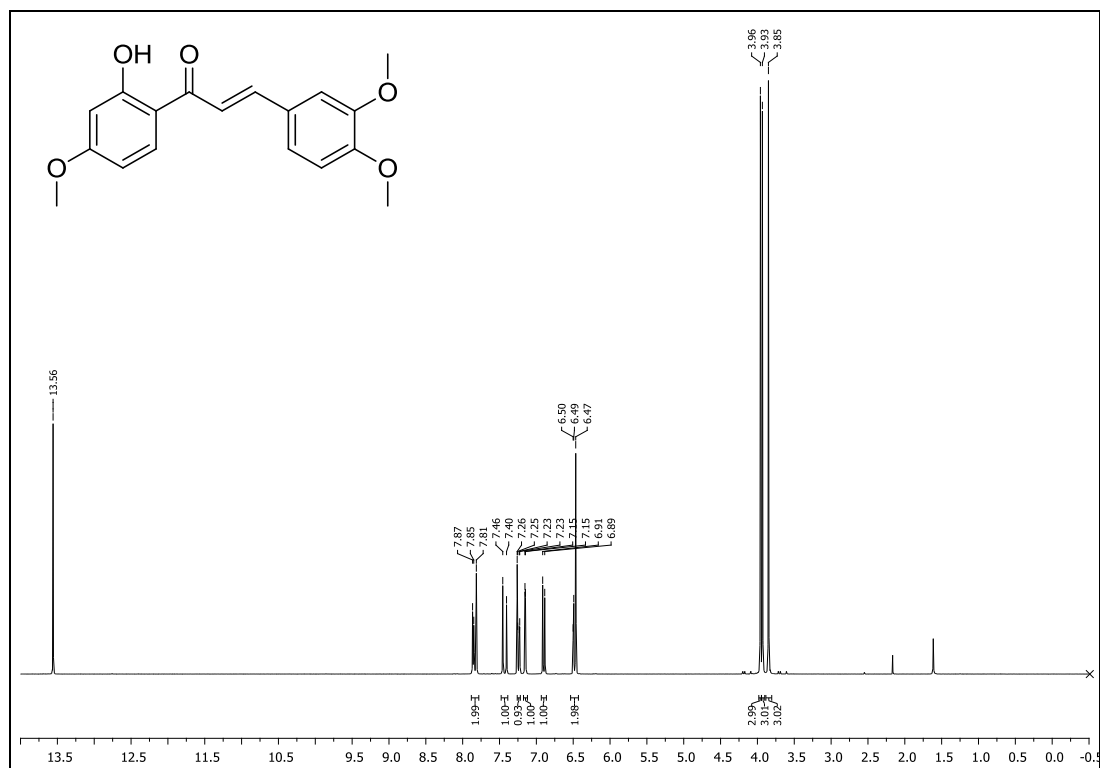
¹³C NMR



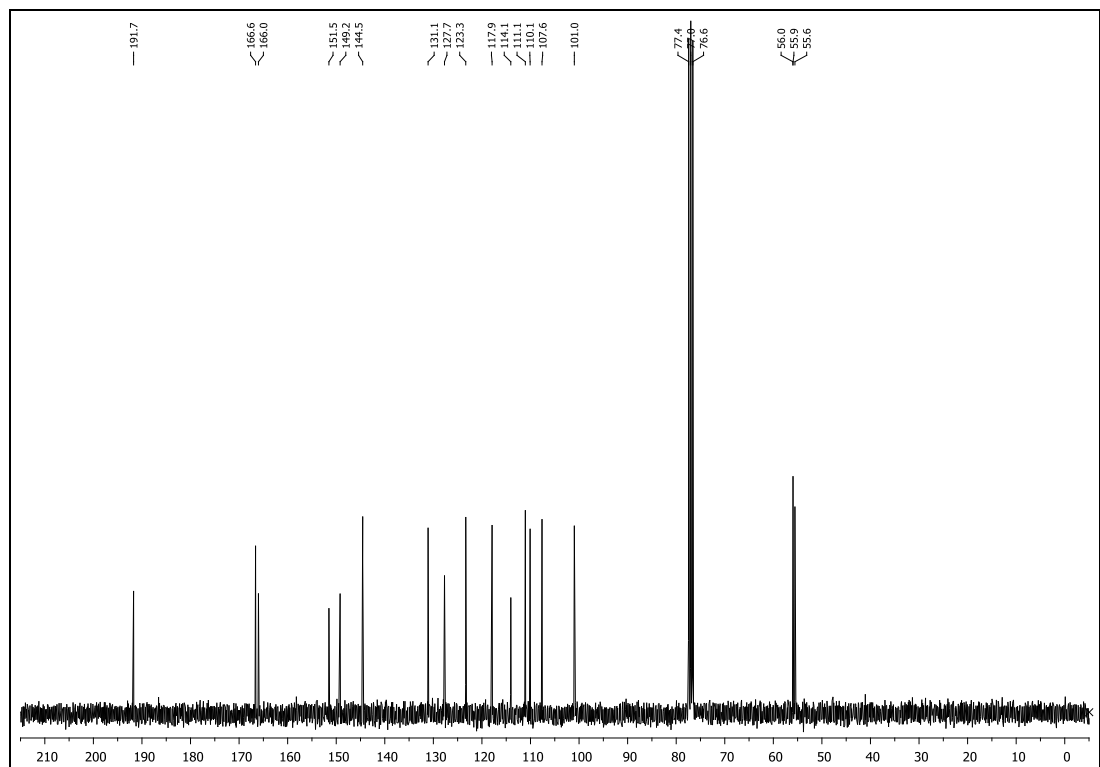
(E)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (α -H-TMC, 60)¹H NMR¹³C NMR

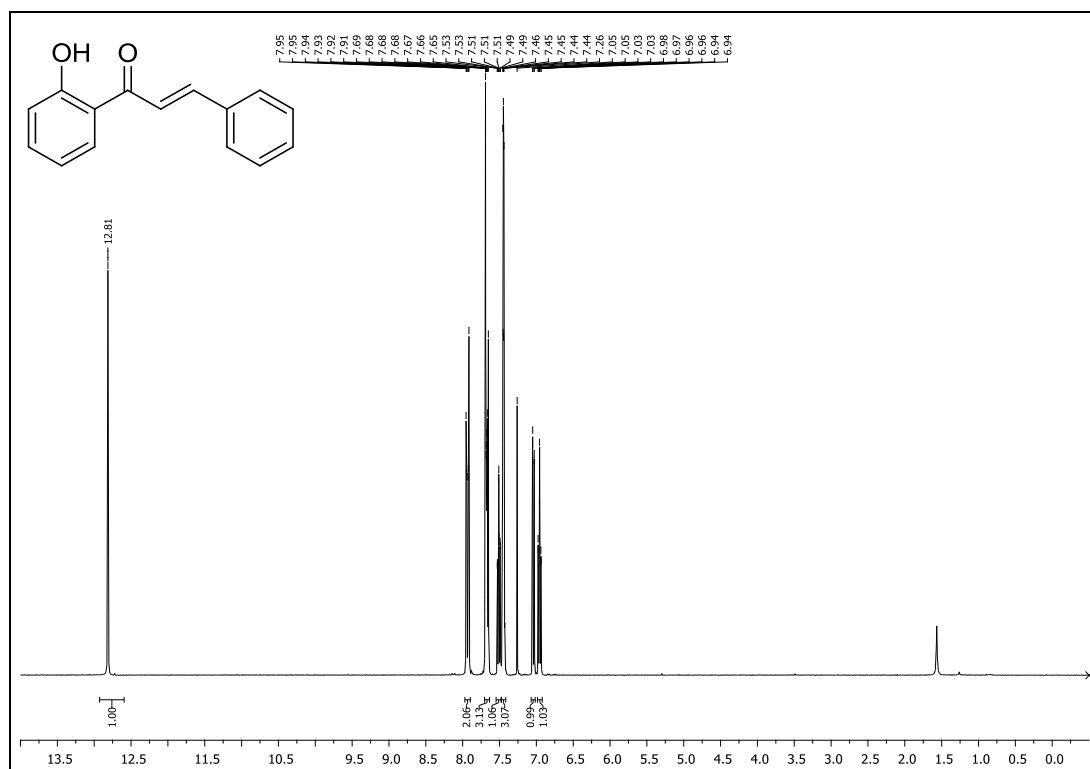
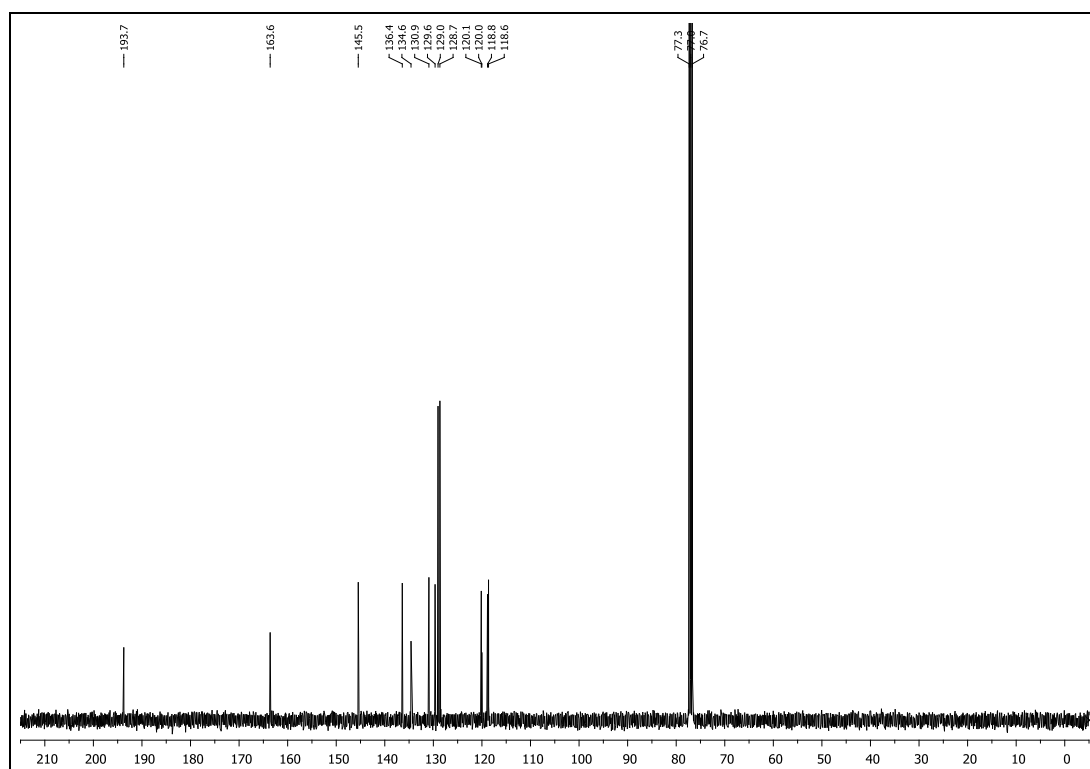
(E)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxy-phenyl)prop-2-en-1-one
(α -H-HC, 61)

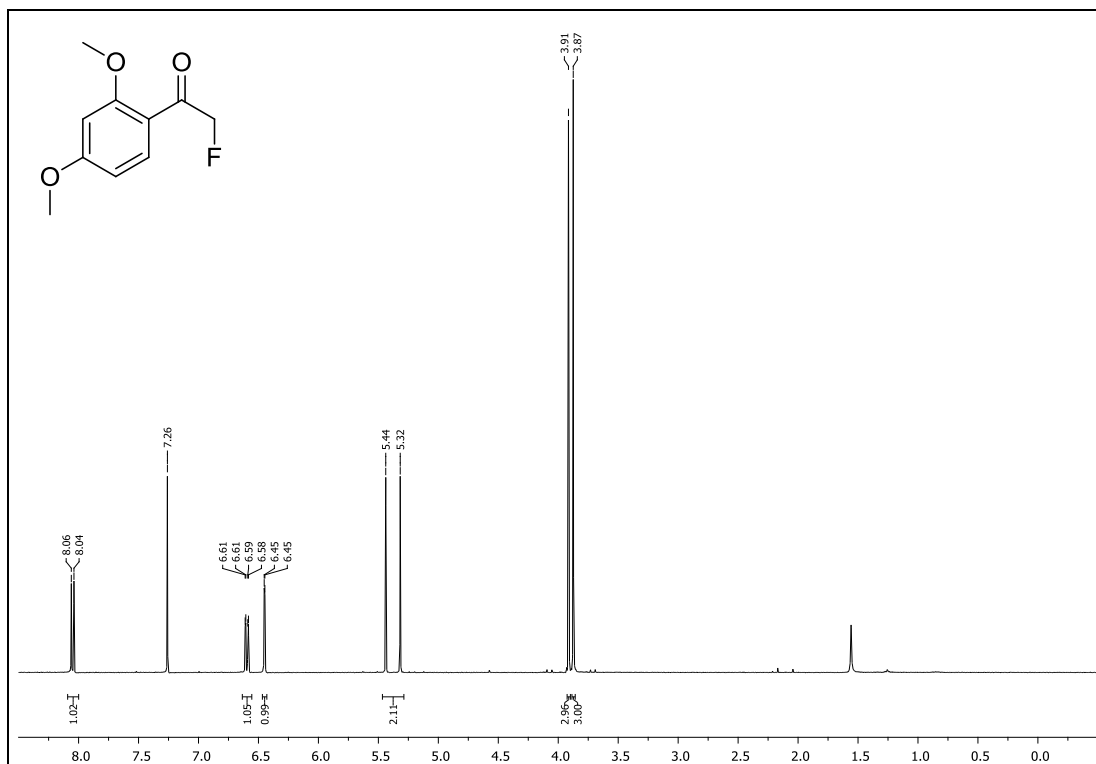
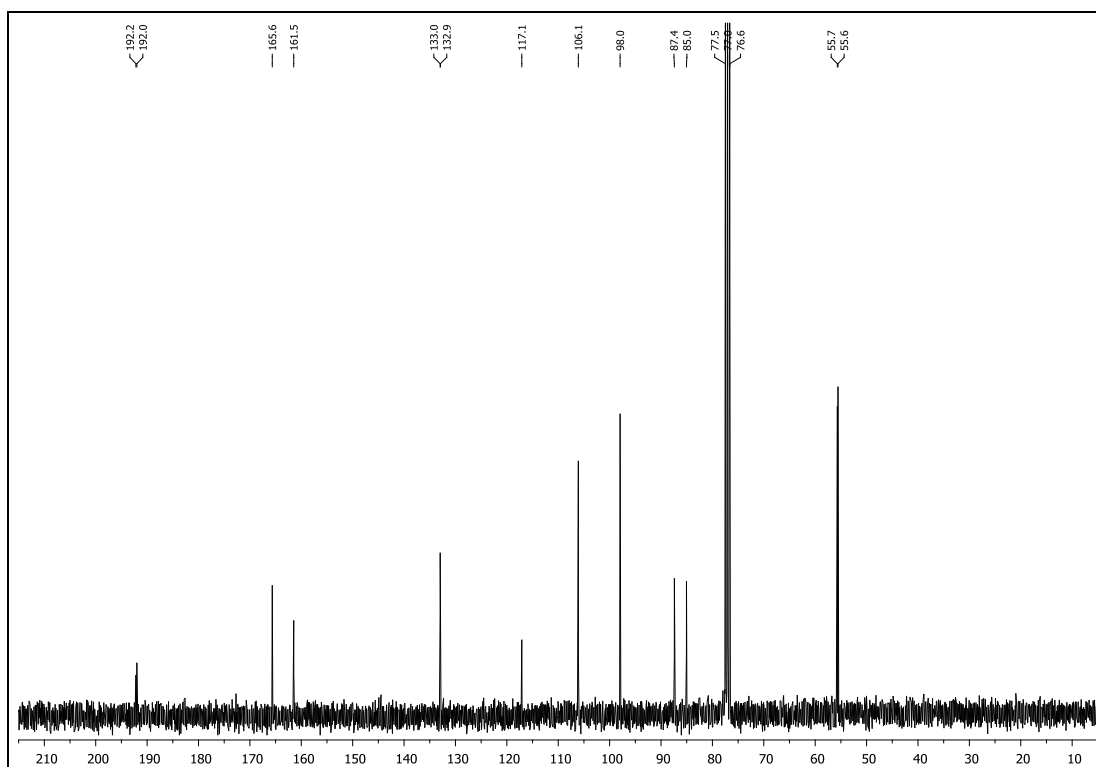
^1H NMR

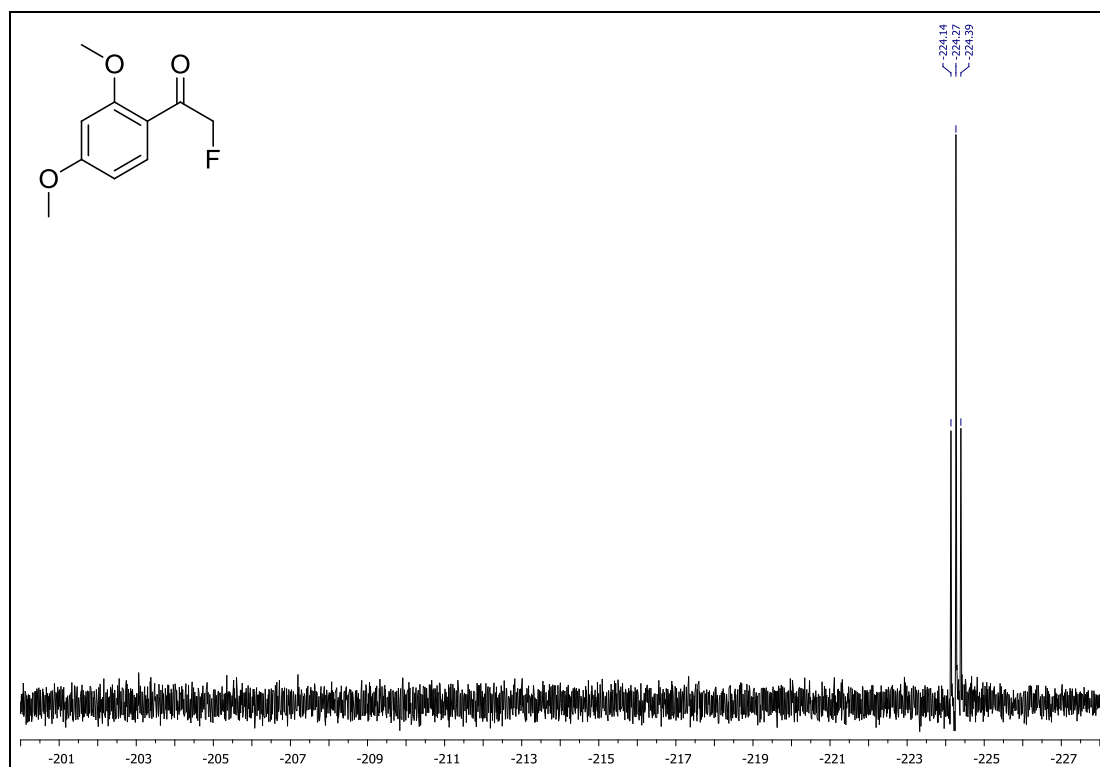


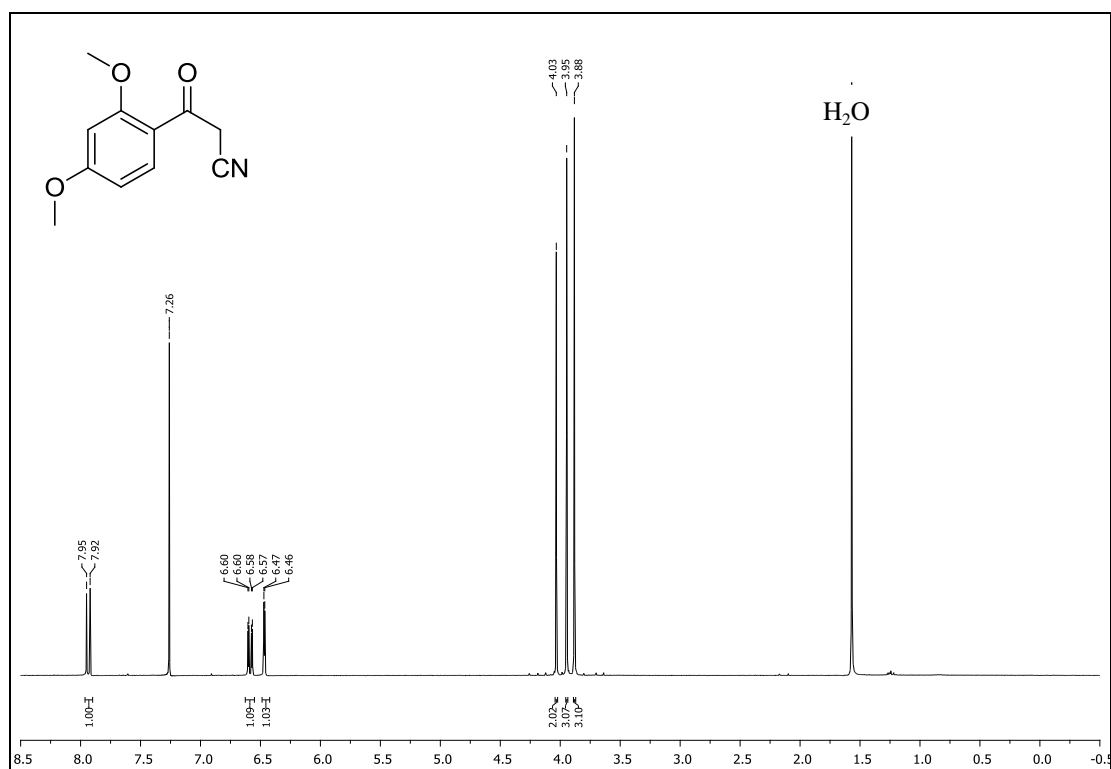
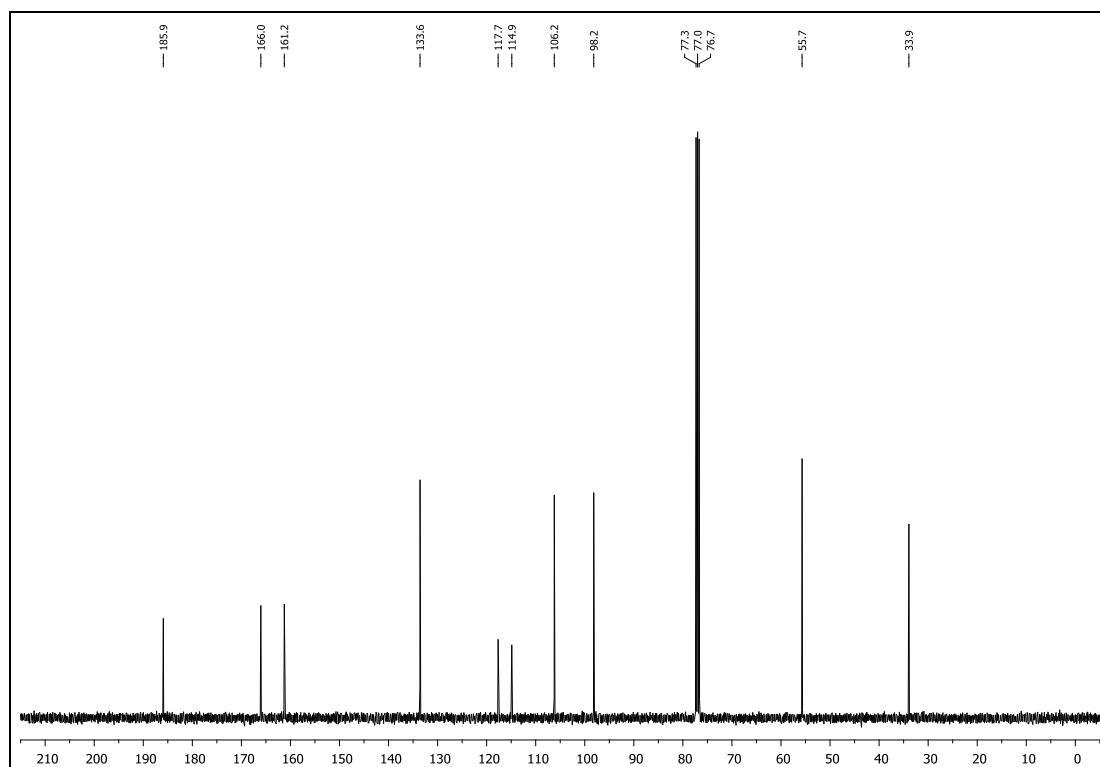
^{13}C NMR

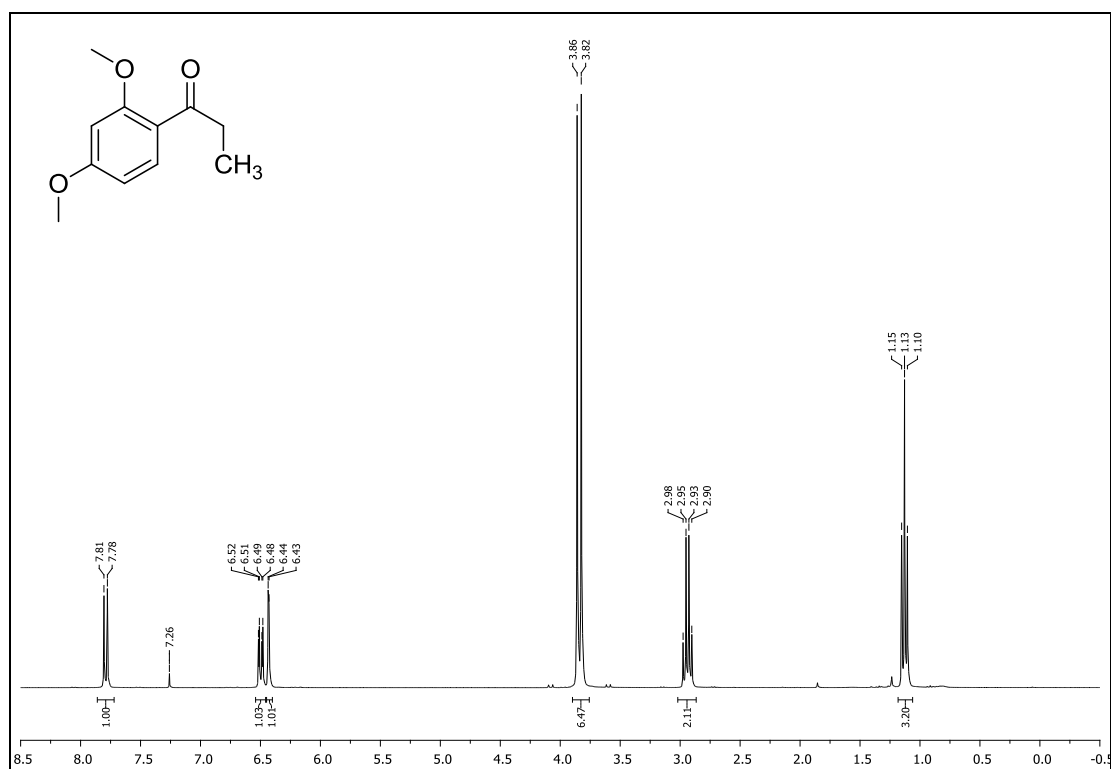
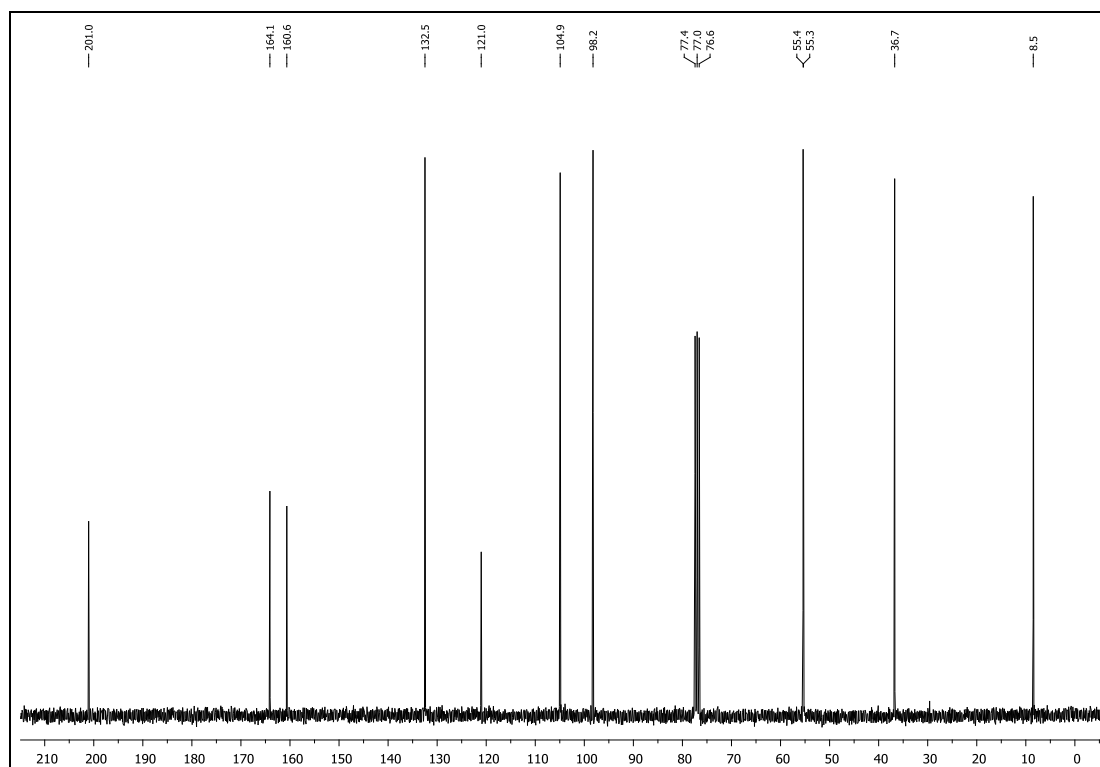


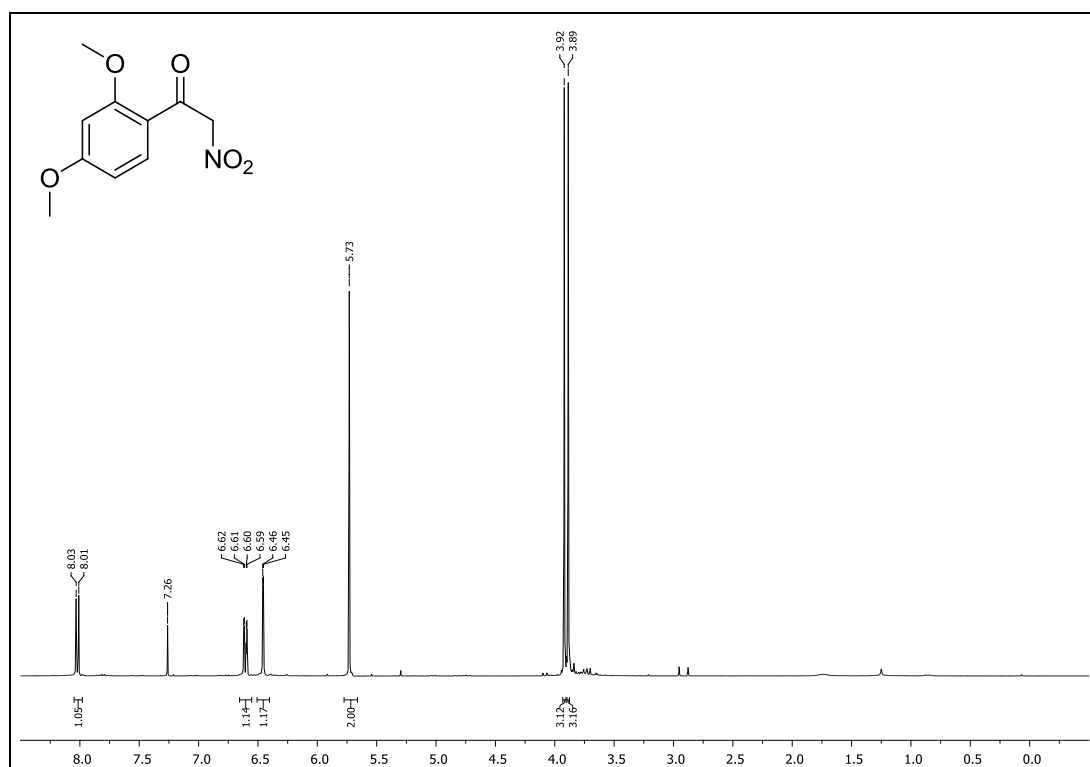
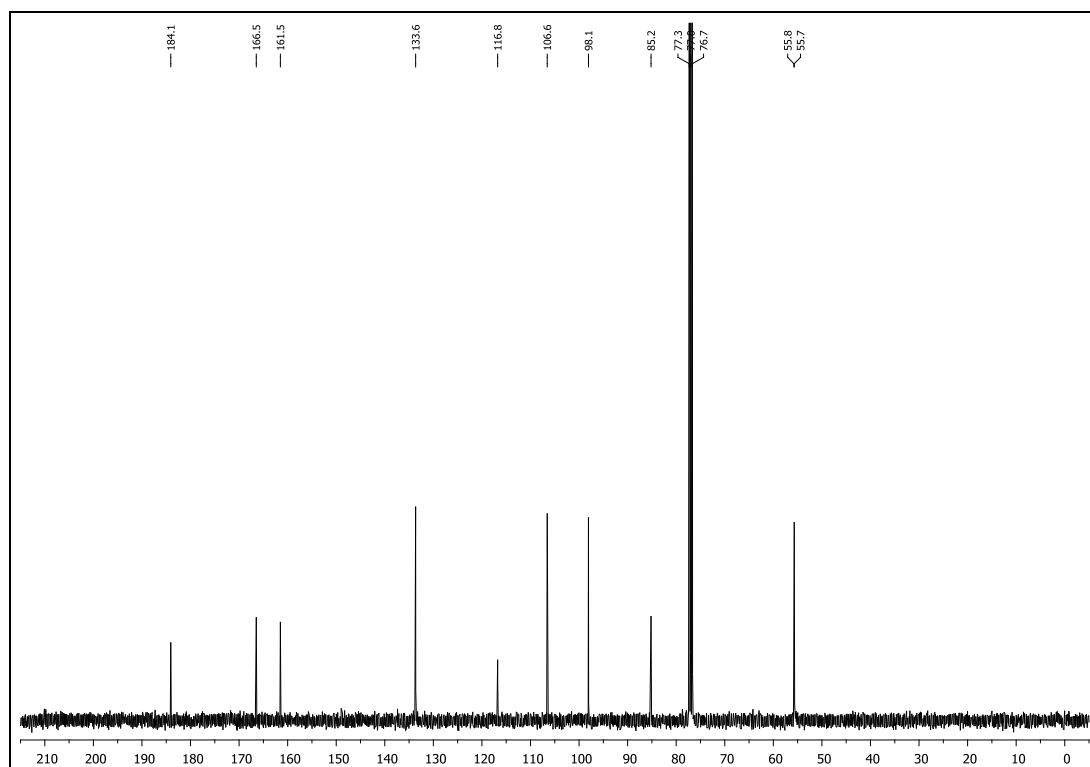
(E)-1-(2-Hydroxyphenyl)-3-phenylprop-2-en-1-one (62)¹H NMR¹³C NMR

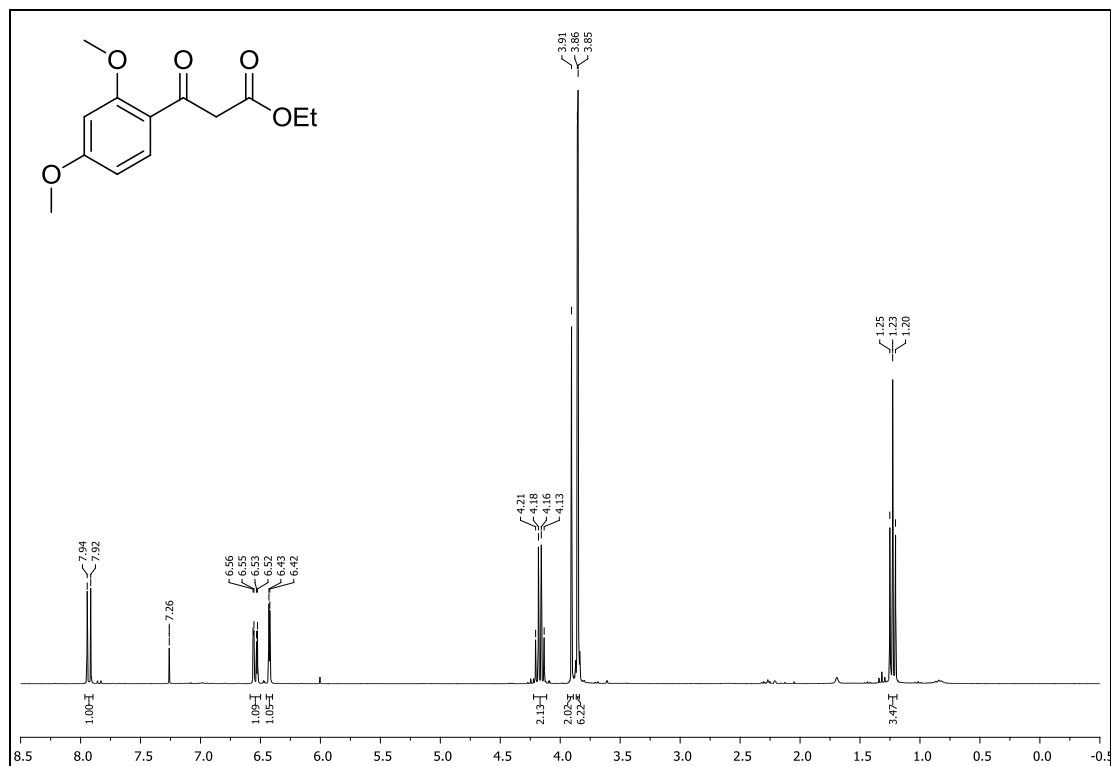
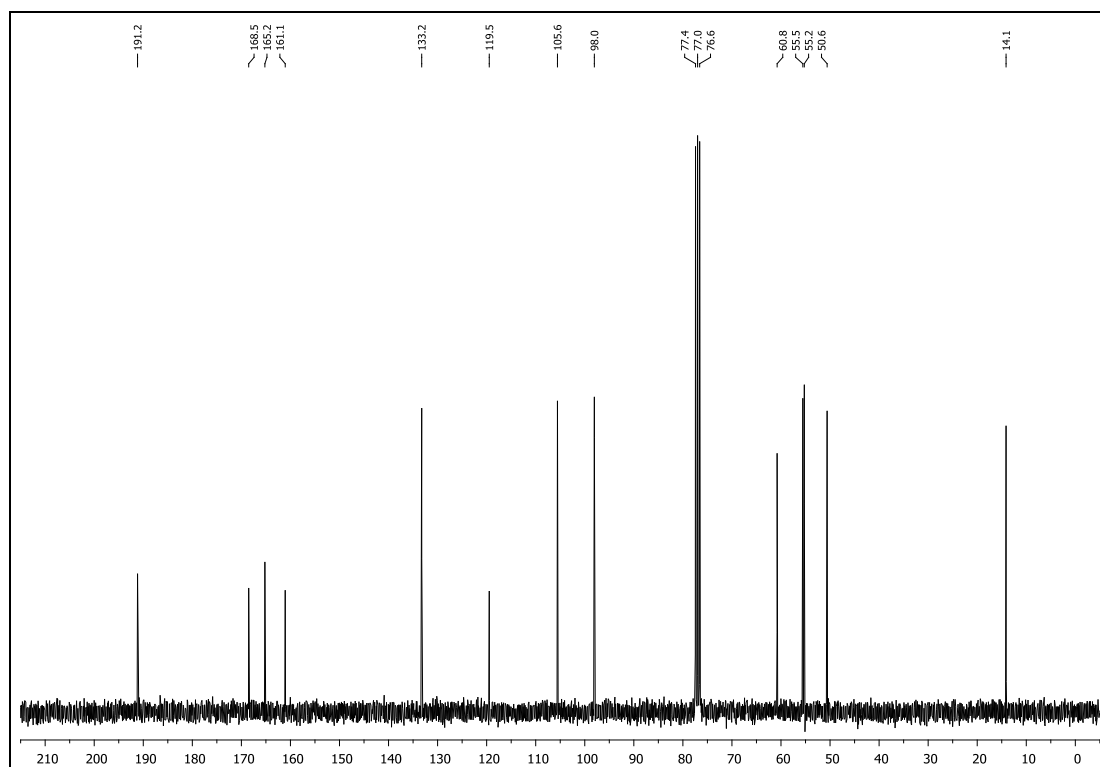
1-(2,4-Dimethoxyphenyl)-2-fluoroethanone (63)¹H NMR¹³C NMR

^{19}F NMR

3-(2,4-Dimethoxyphenyl)-3-oxopropanenitrile (64)¹H NMR¹³C NMR

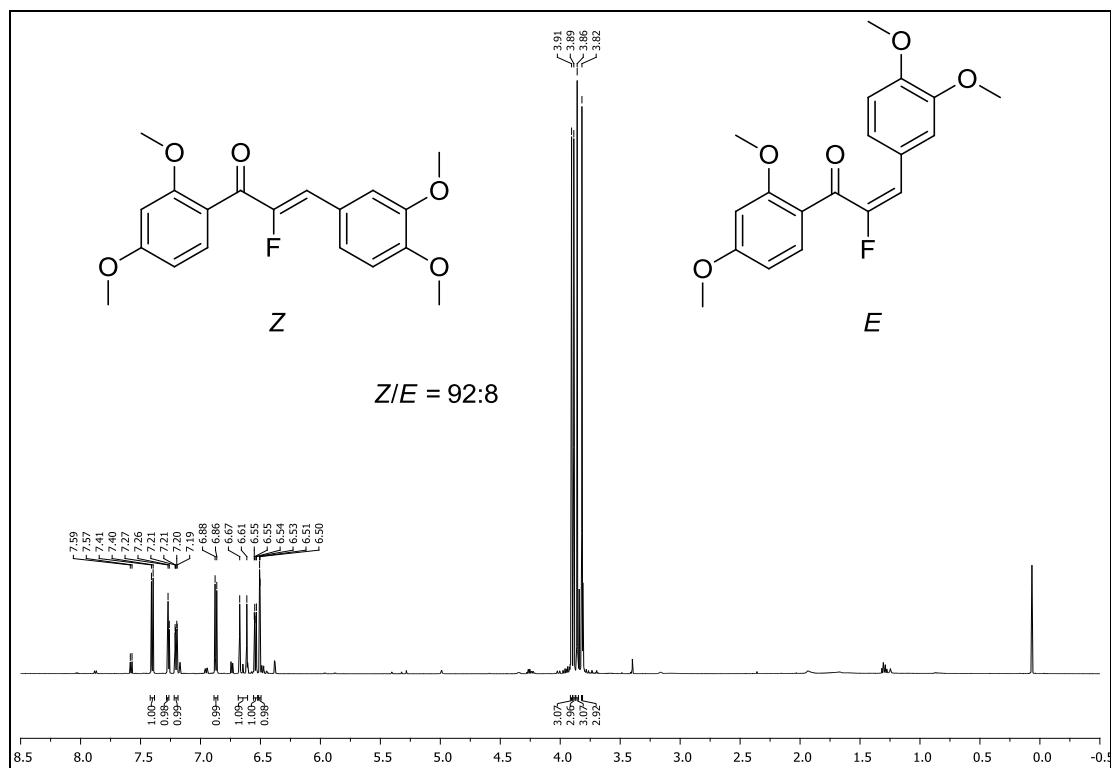
1-(2,4-Dimethoxyphenyl)propan-1-one (65)¹H NMR¹³C NMR

1-(2,4-Dimethoxyphenyl)-2-nitroethanone (66)¹H NMR¹³C NMR

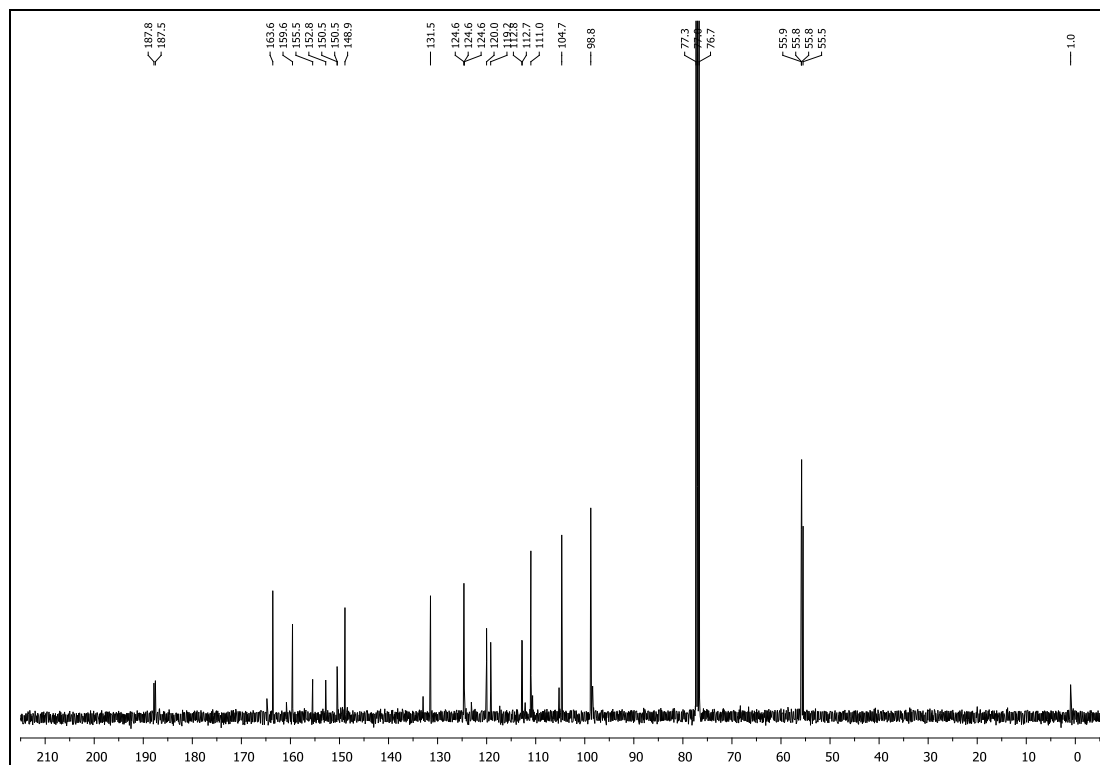
Ethyl 3-(2,4-dimethoxyphenyl)-3-oxopropanoate (67)¹H NMR¹³C NMR

(*Z/E*)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-fluoroprop-2-en-1-one
(α -F-TMC, 68)

^1H NMR



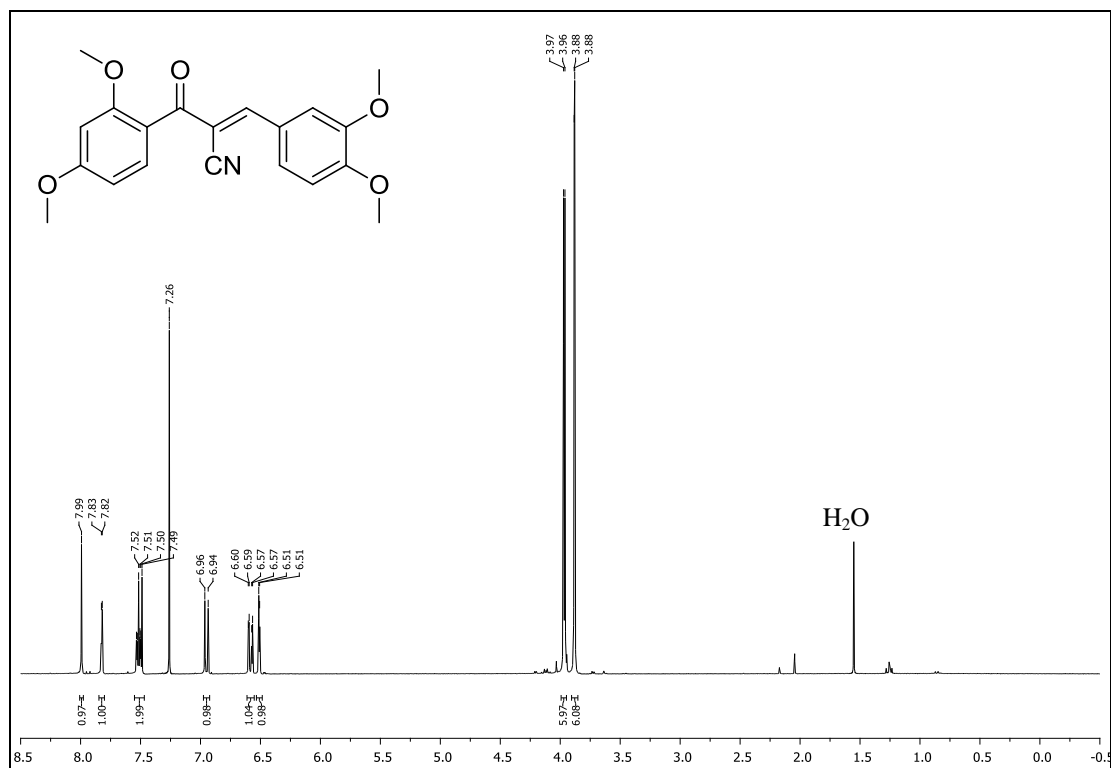
^{13}C NMR



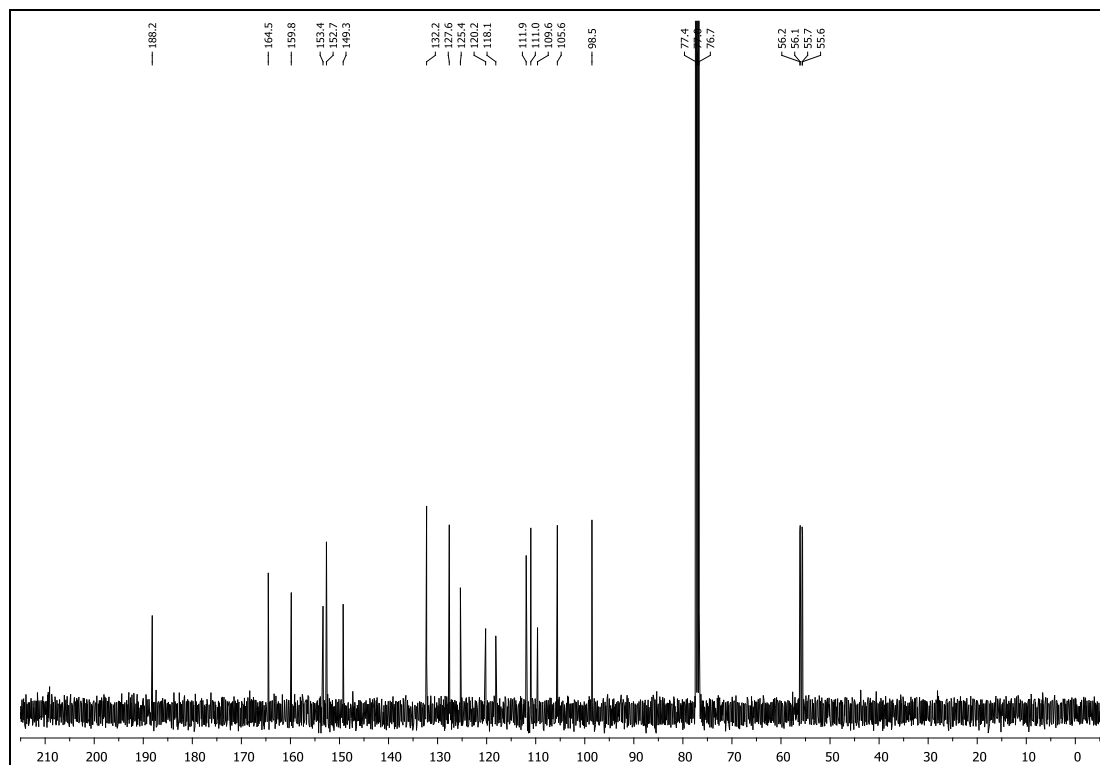
^{19}F NMR

(*E*)-2-Cyano-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one
(α -CN-TMC, 69)

^1H NMR

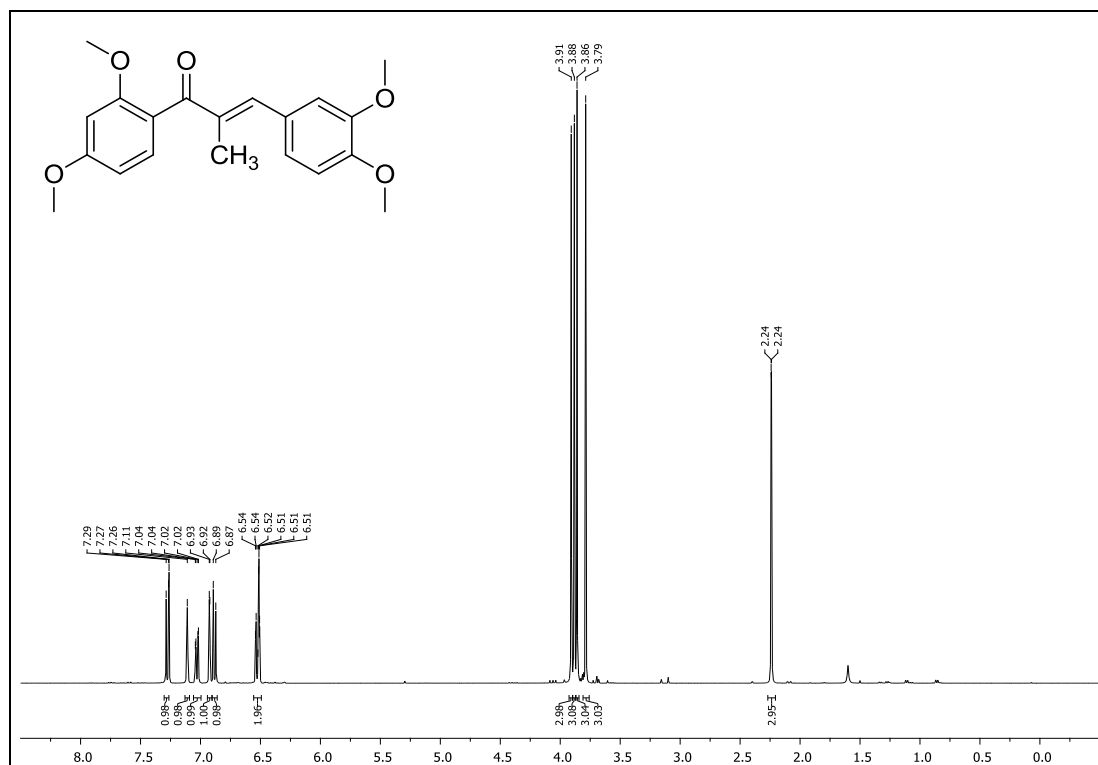


^{13}C NMR

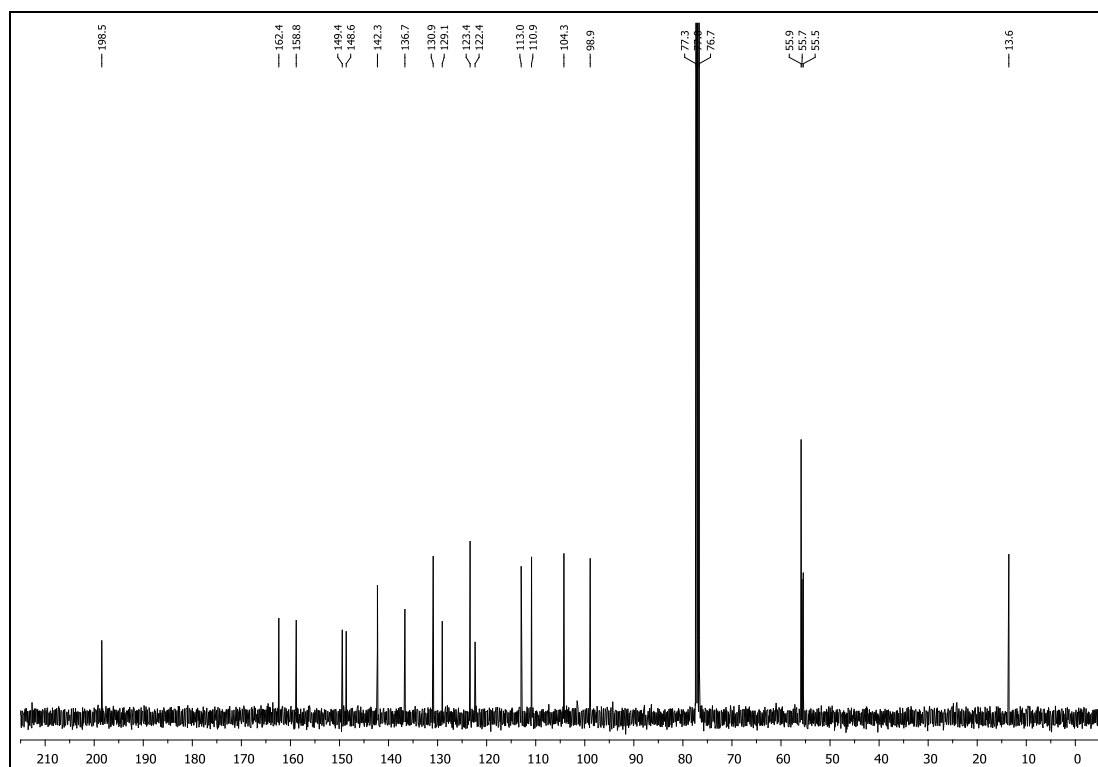


(*E*)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-methylprop-2-en-1-one
(α -Me-TMC, 70)

^1H NMR

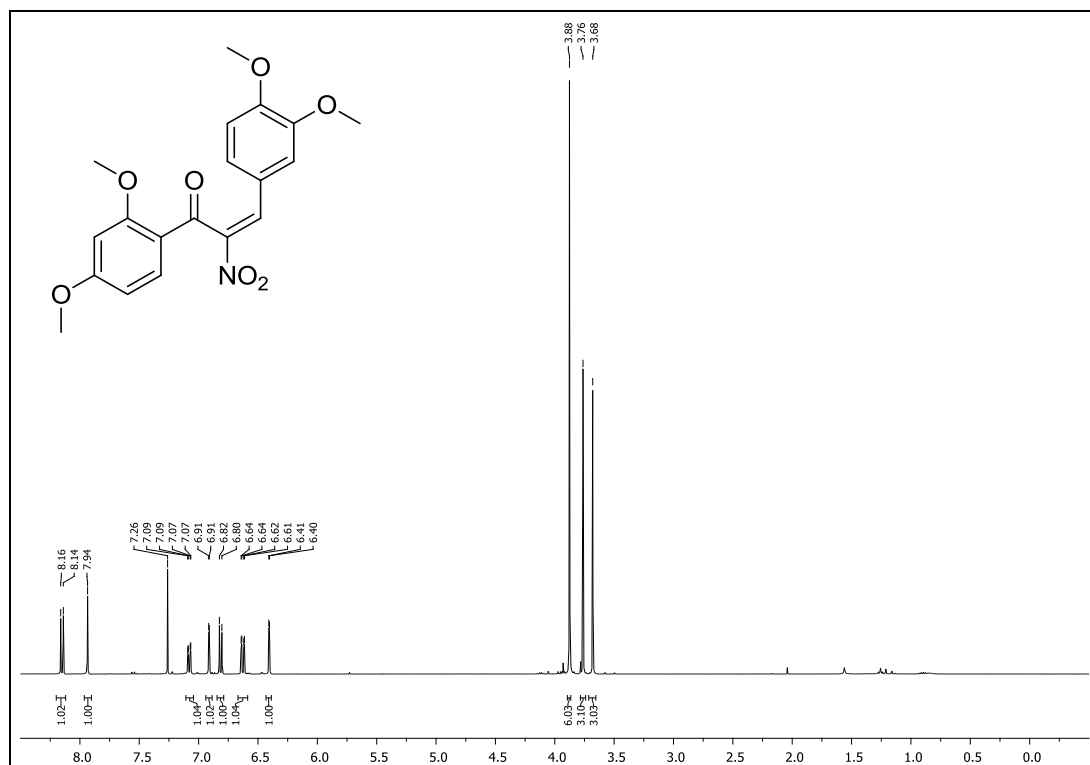


^{13}C NMR

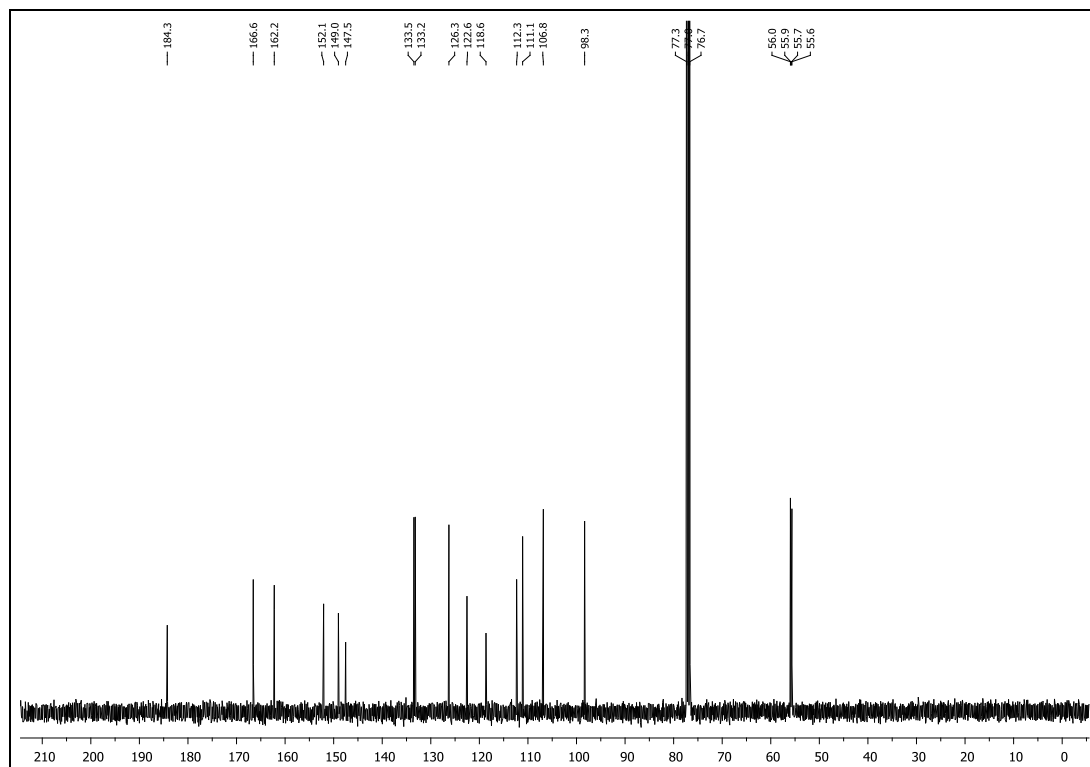


(E)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-nitroprop-2-en-1-one
(α -NO₂-TMC, 71)

¹H NMR

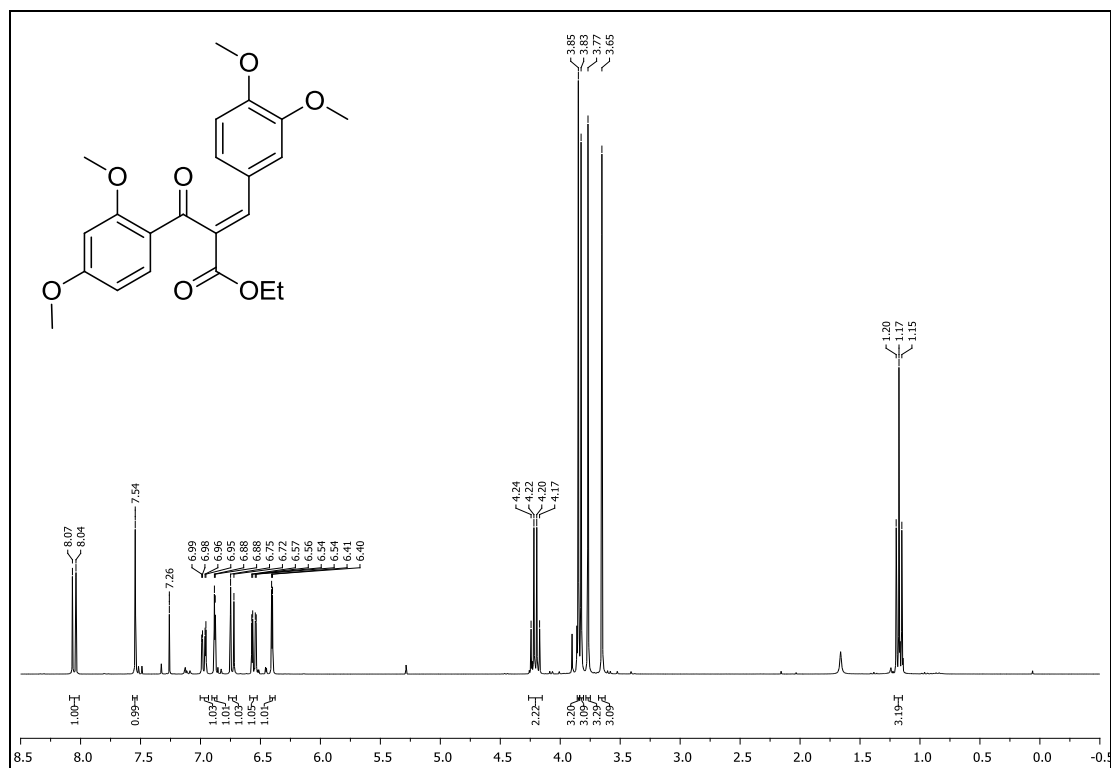


¹³C NMR

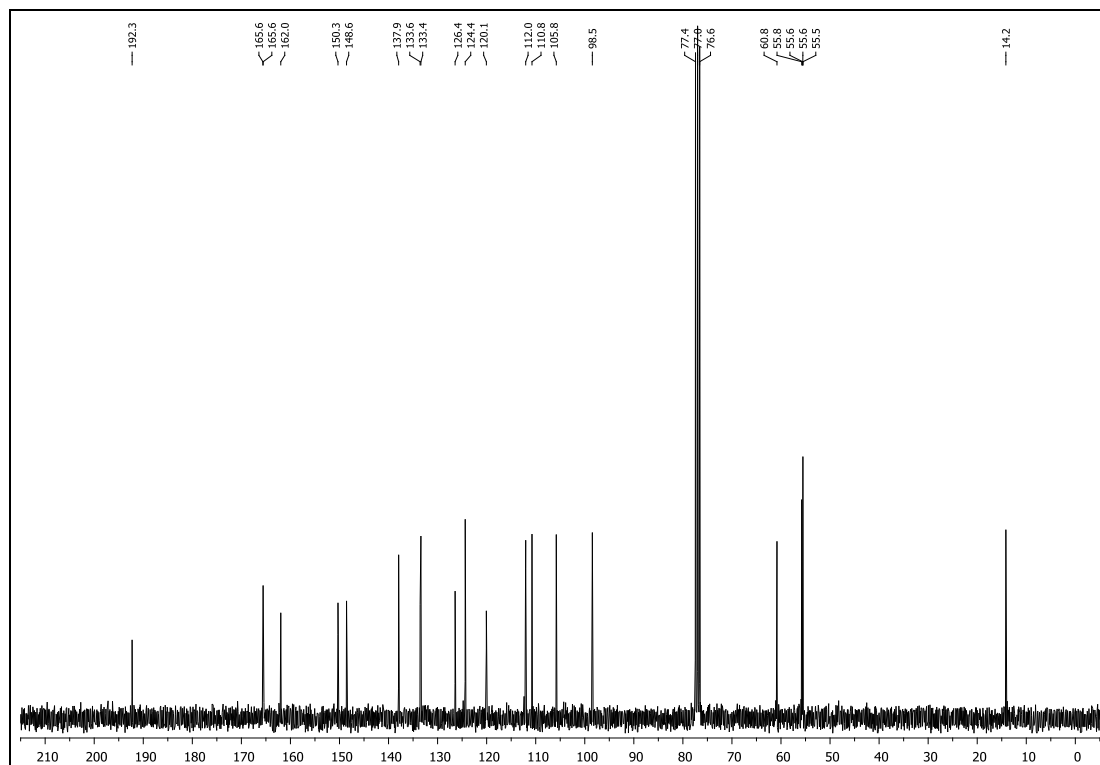


(*E*)-2-Ethoxycarbonyl-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)propen-1-one
(α -COOEt-TMC, 72)

¹H NMR

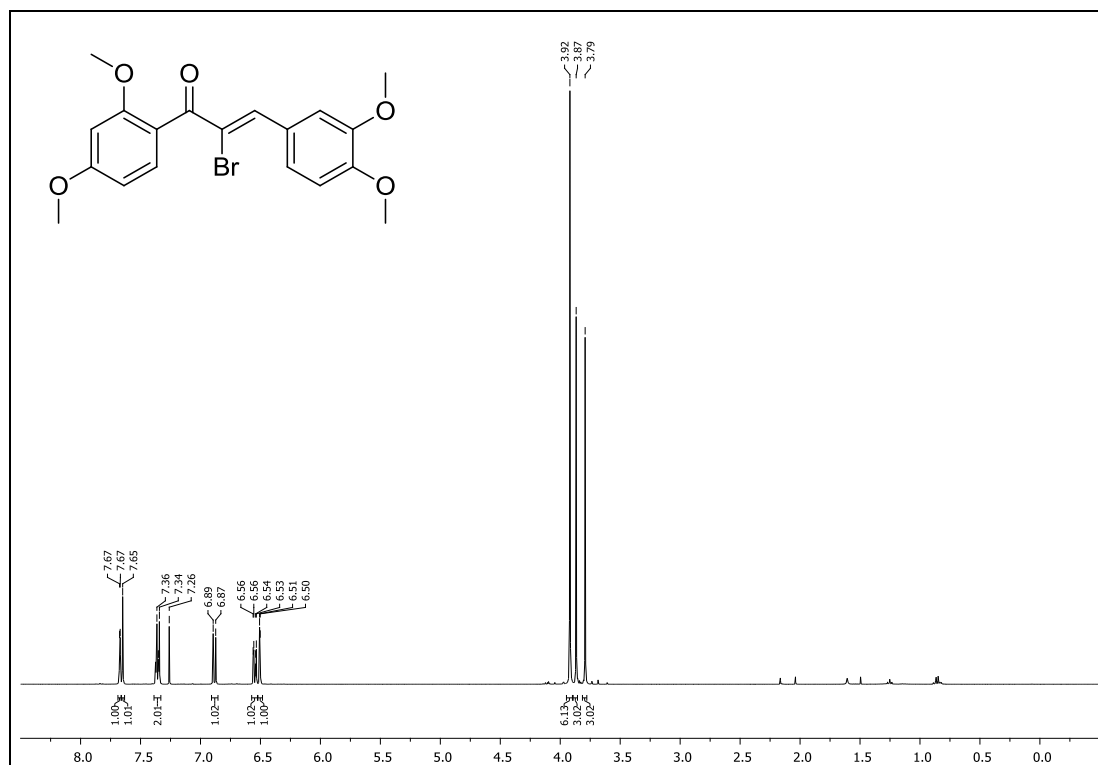


¹³C NMR

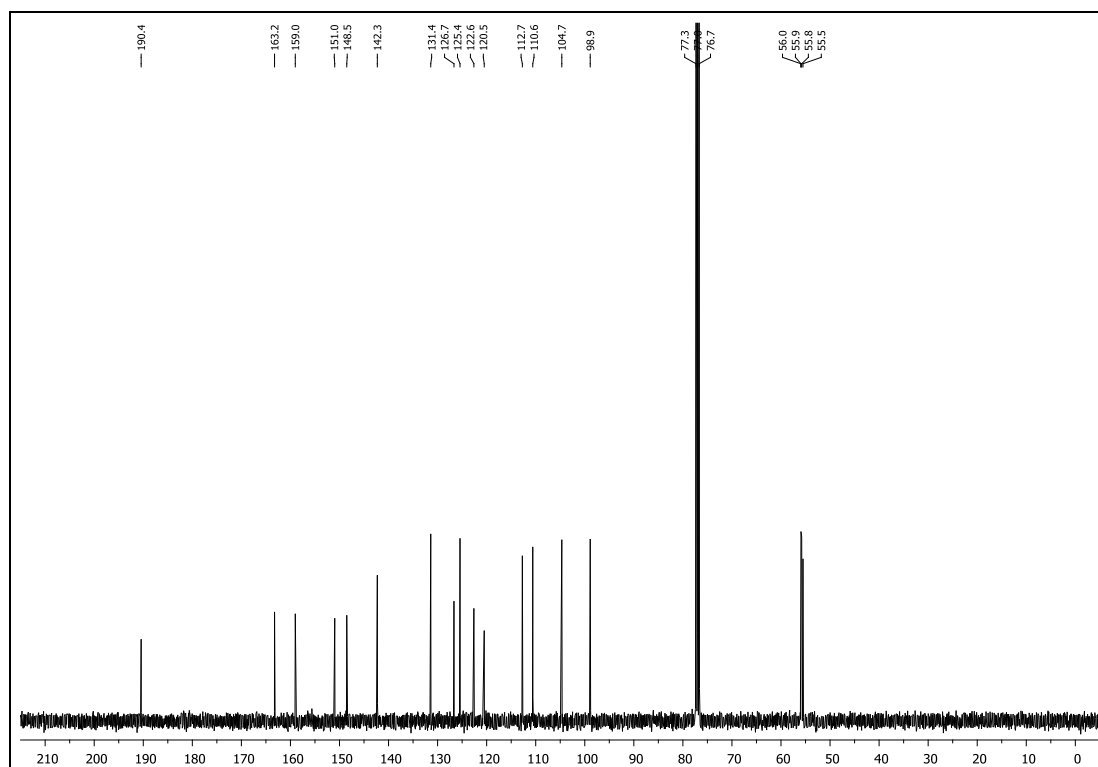


(Z)-2-Bromo-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxy-phenyl)prop-2-en-1-one
(α -Br-TMC, 73)

^1H NMR

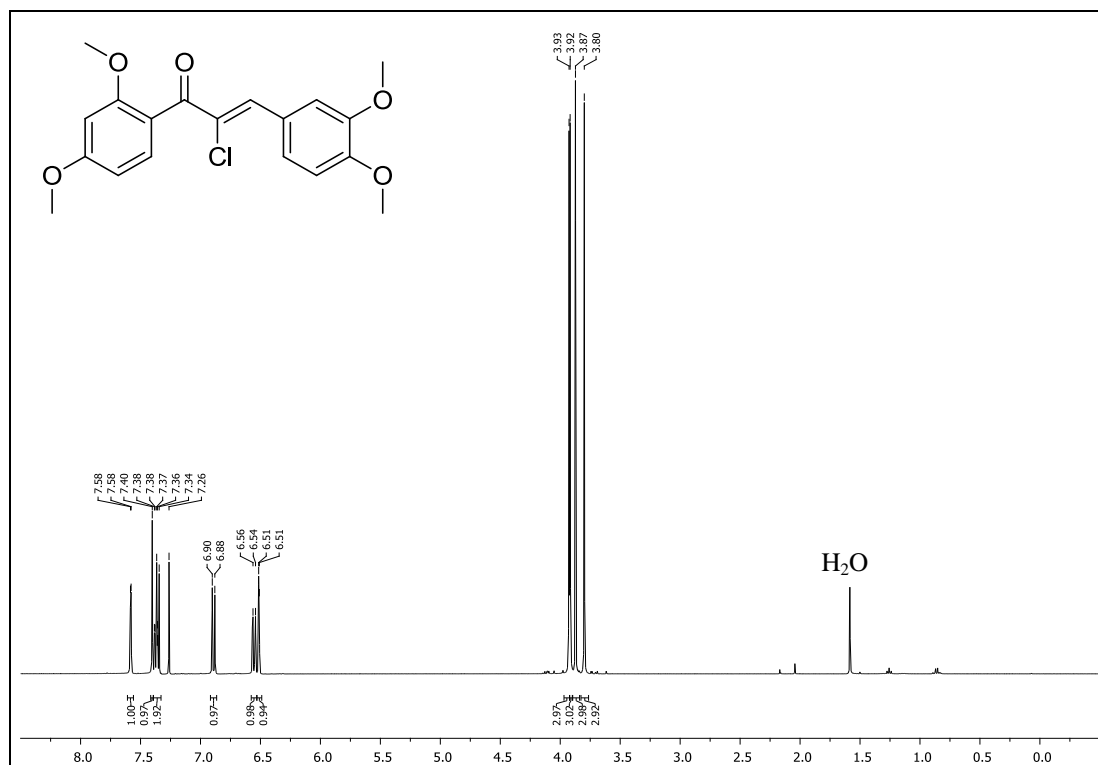


^{13}C NMR

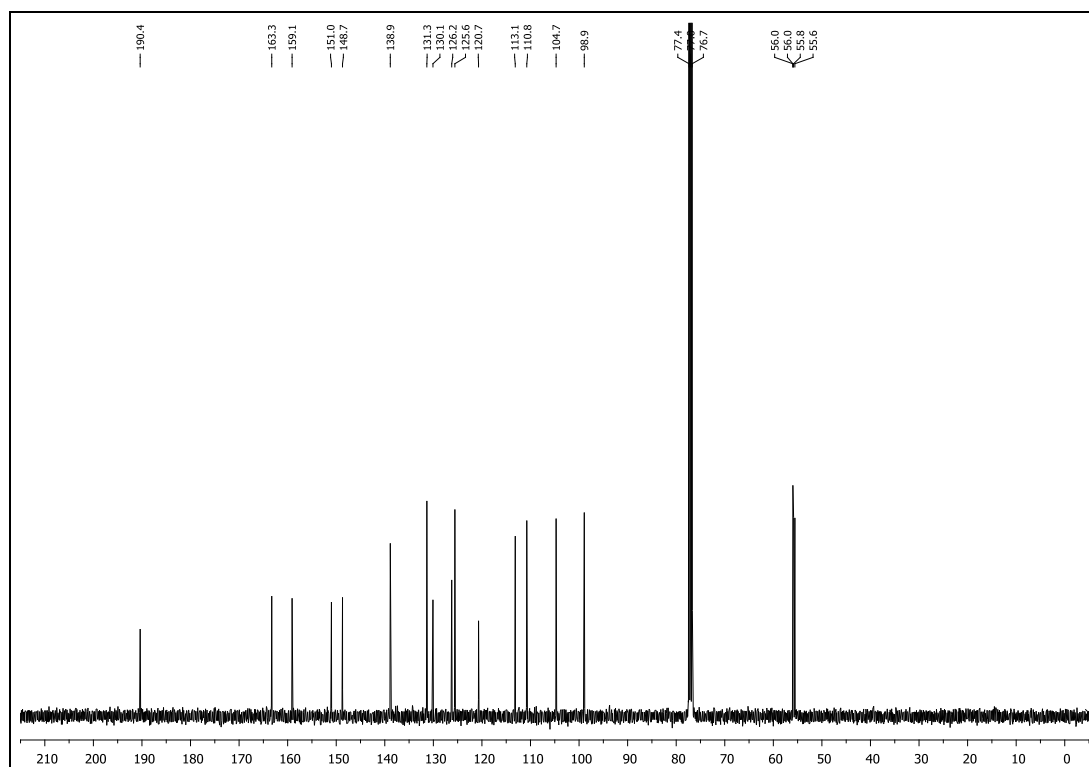


(Z)-2-Chloro-1-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxy-phenyl)prop-2-en-1-one
(α -Cl-TMC, 74)

^1H NMR

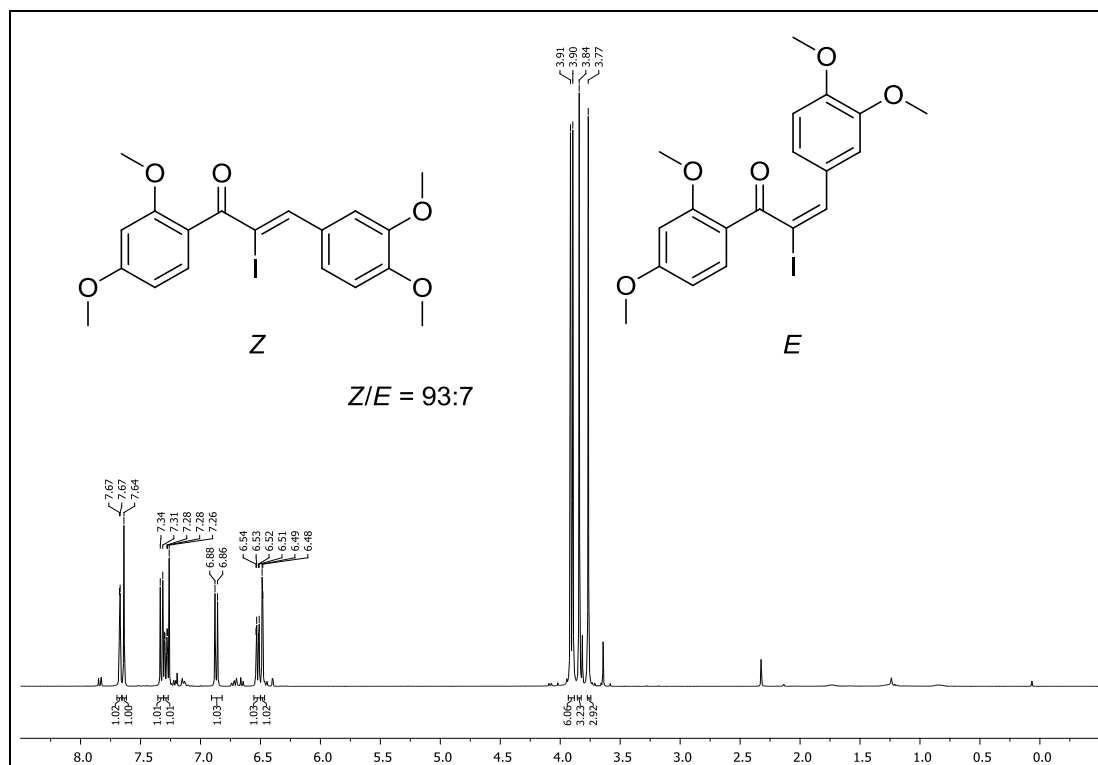


^{13}C NMR

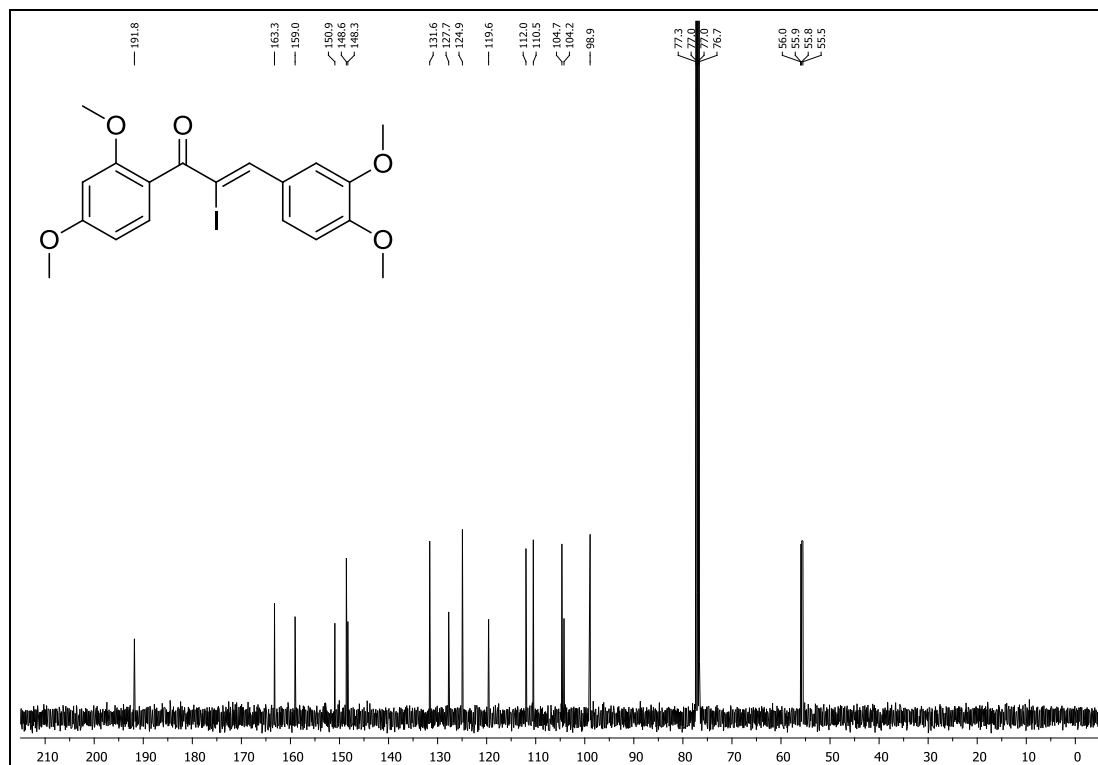


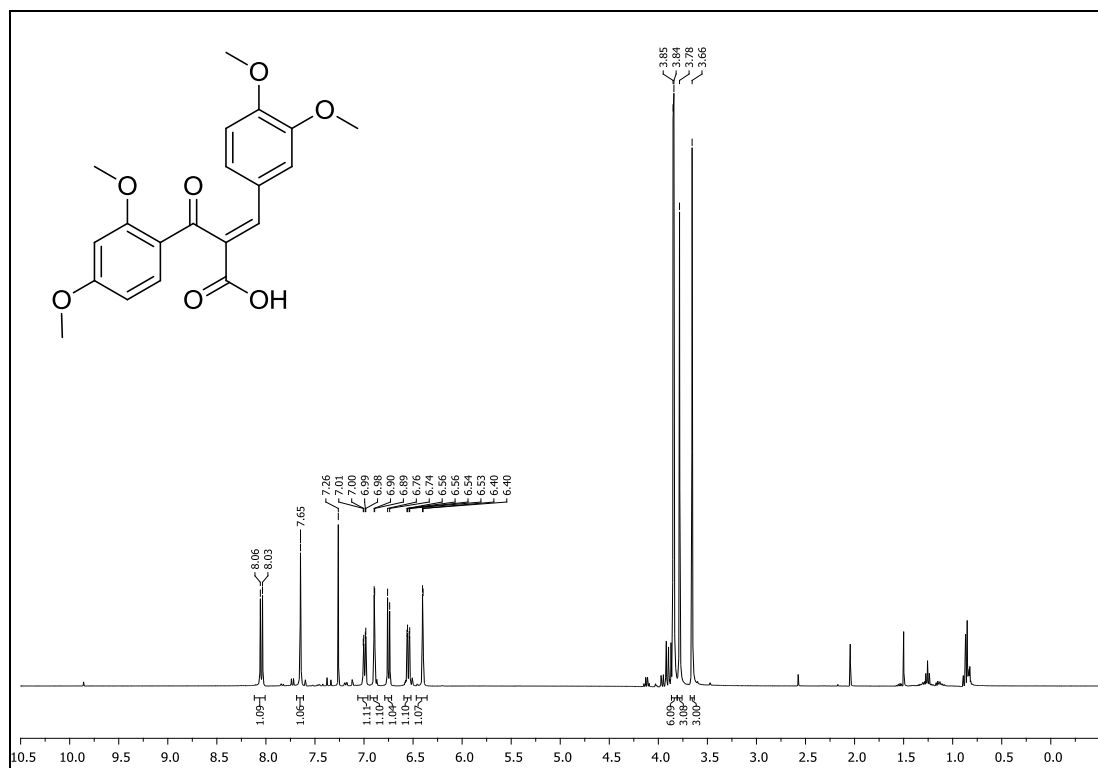
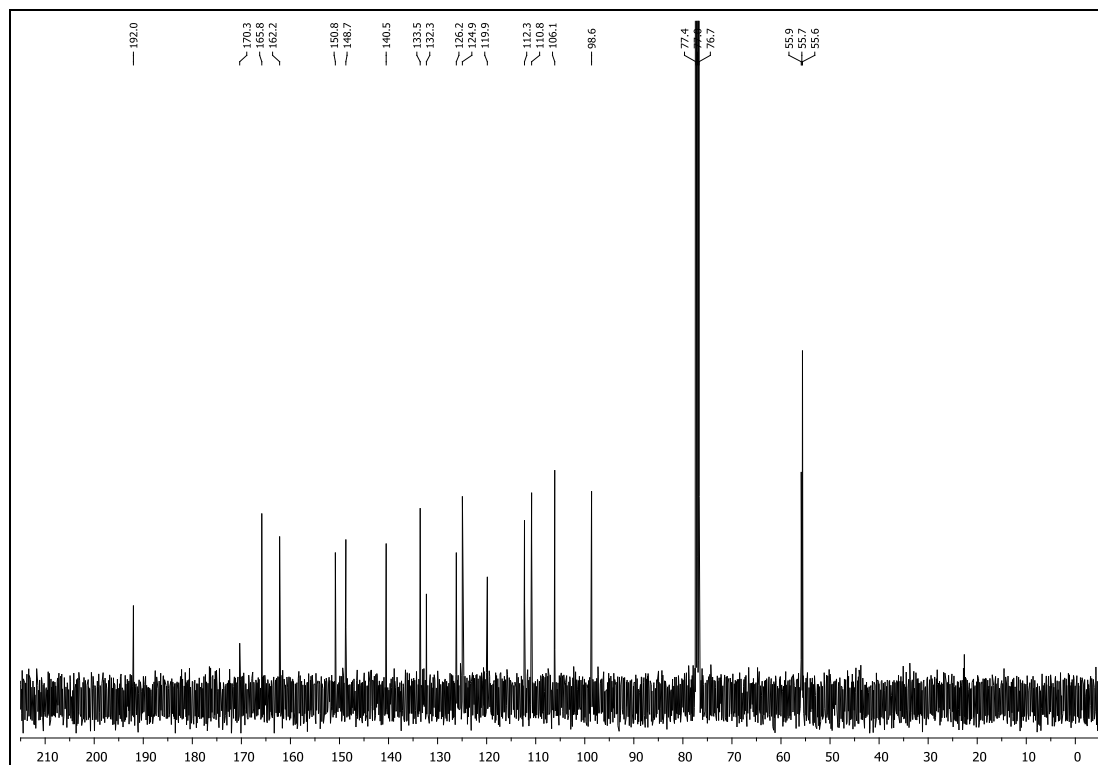
(*Z/E*)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-iodoprop-2-en-1-one
(α -I-TMC, 75)

^1H NMR



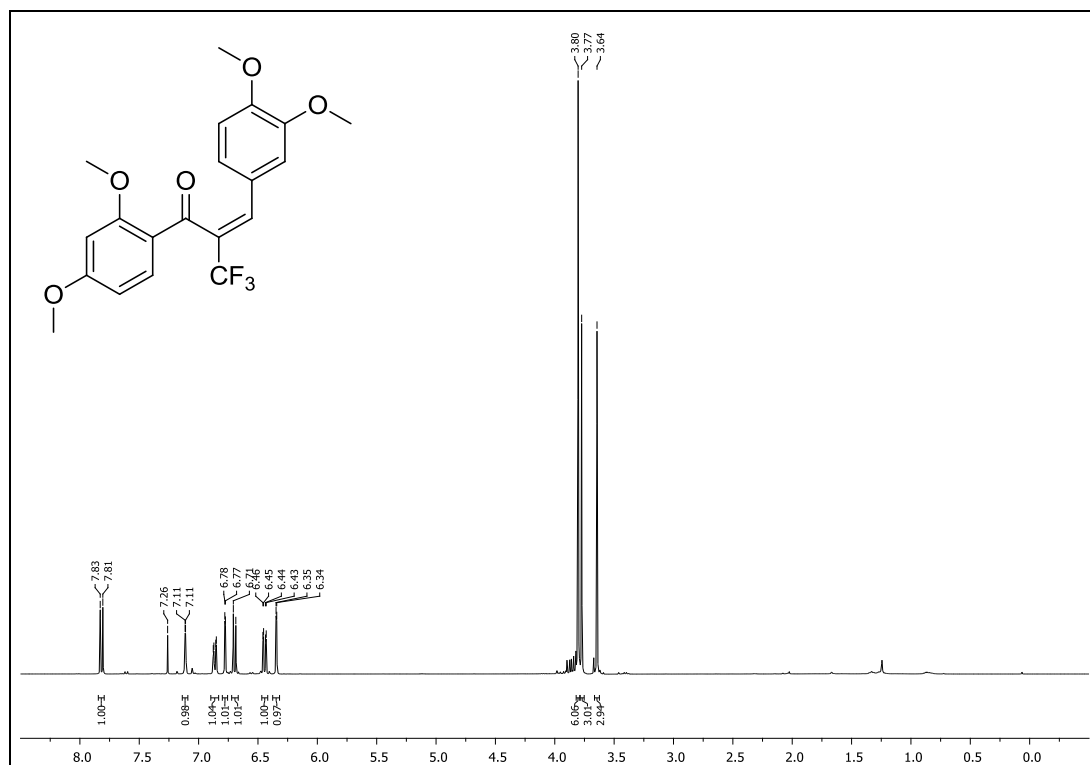
^{13}C NMR



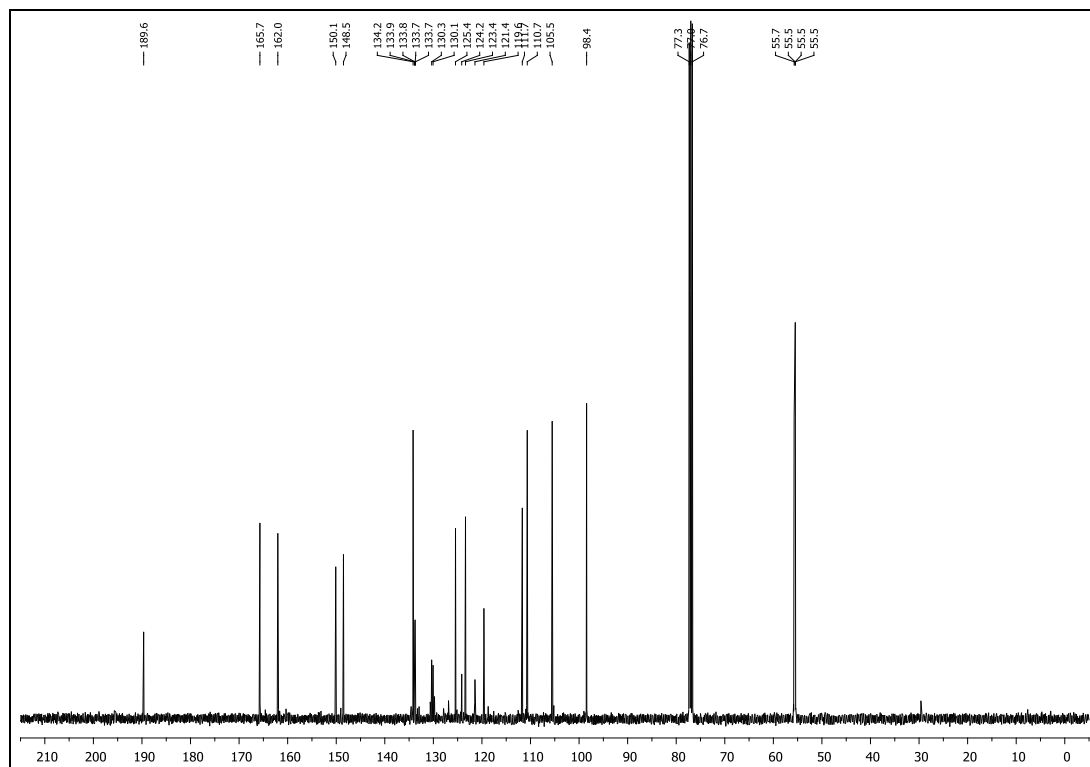
(E)-2-(2,4-Dimethoxybenzoyl)-3-(3,4-dimethoxyphenyl)propenoic acid (α -COOH-TMC, 76)¹H NMR¹³C NMR

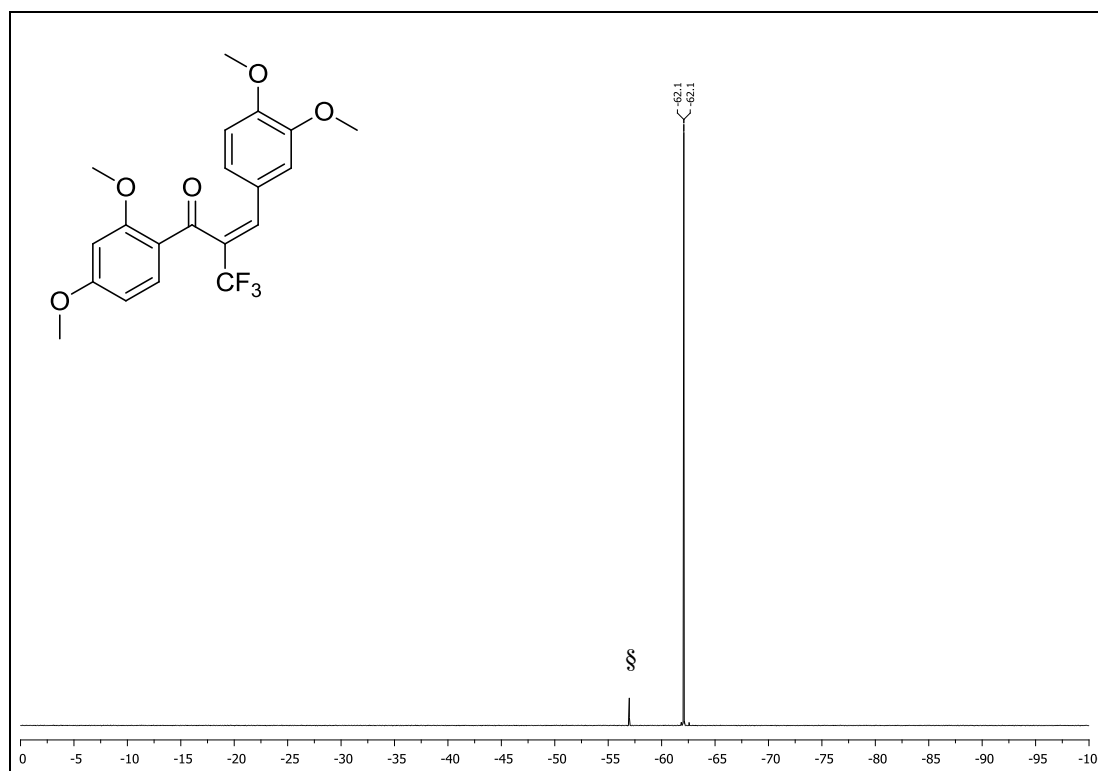
(*E*)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-(trifluoromethyl)prop-2-en-1-one (α -CF₃-TMC, 77)

¹H NMR

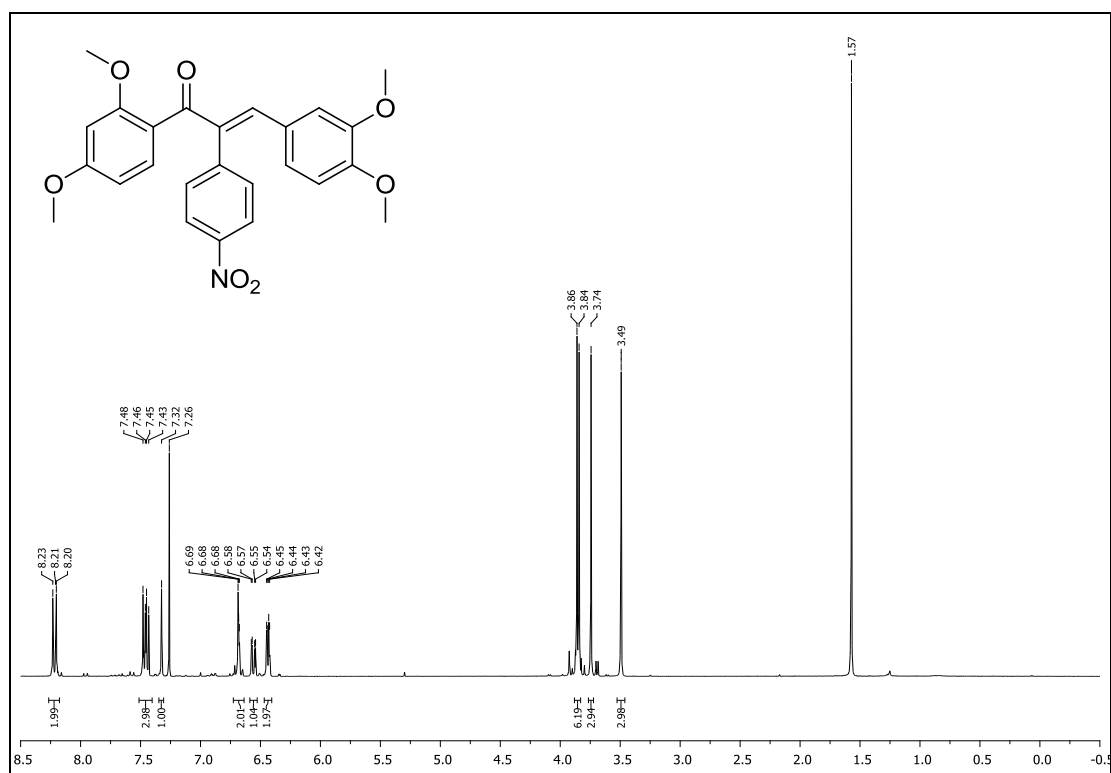
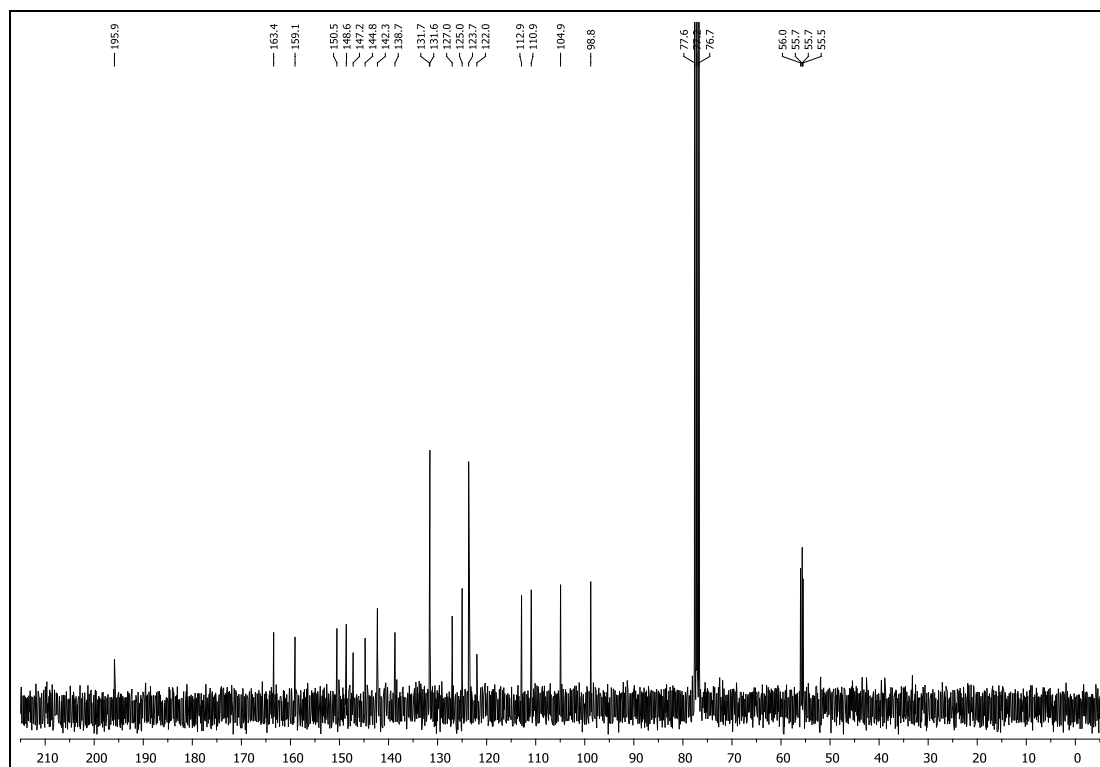


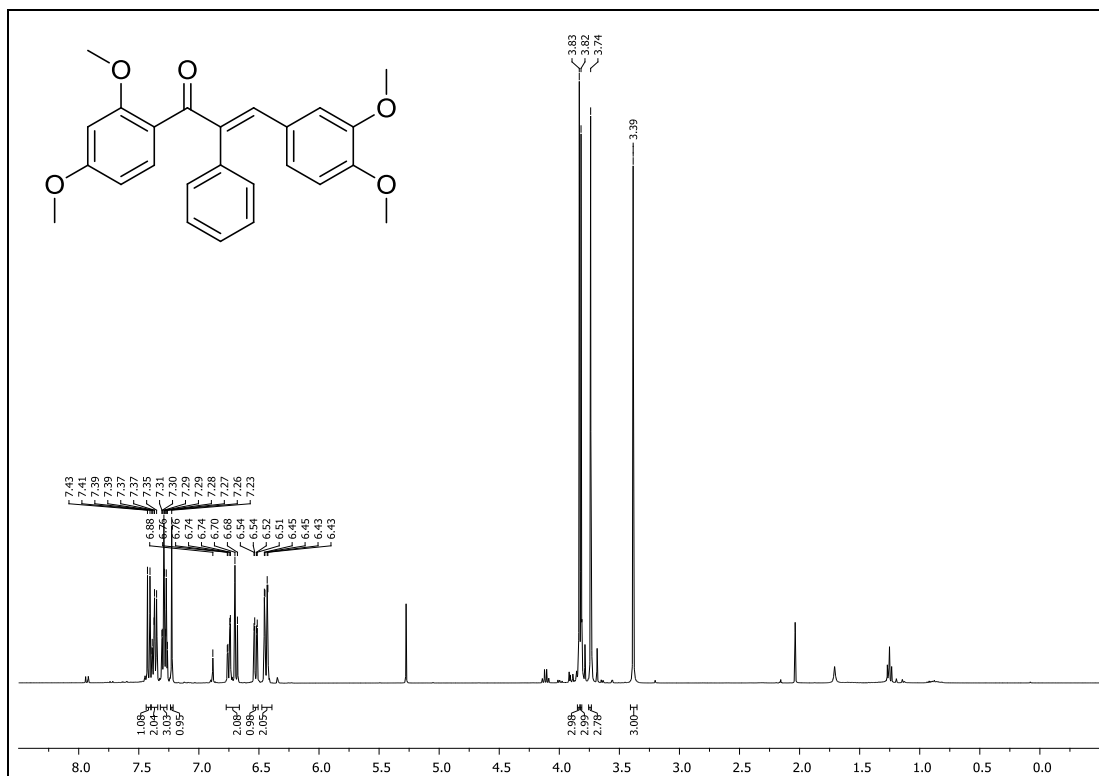
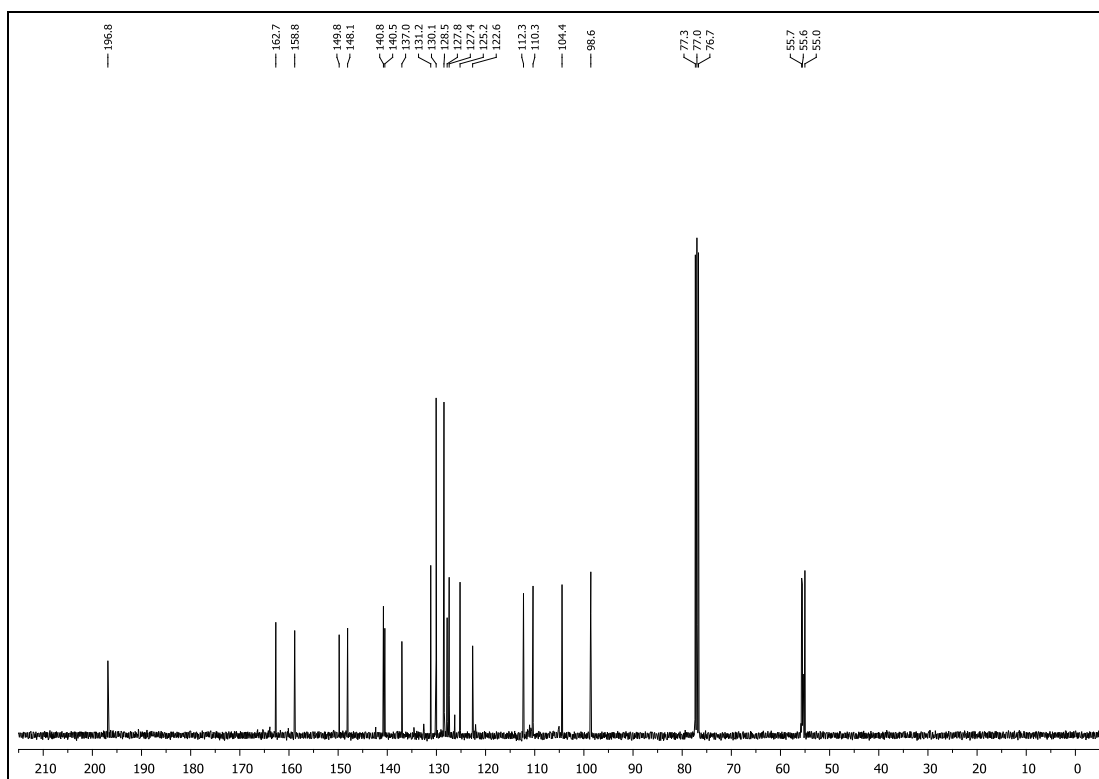
¹³C NMR

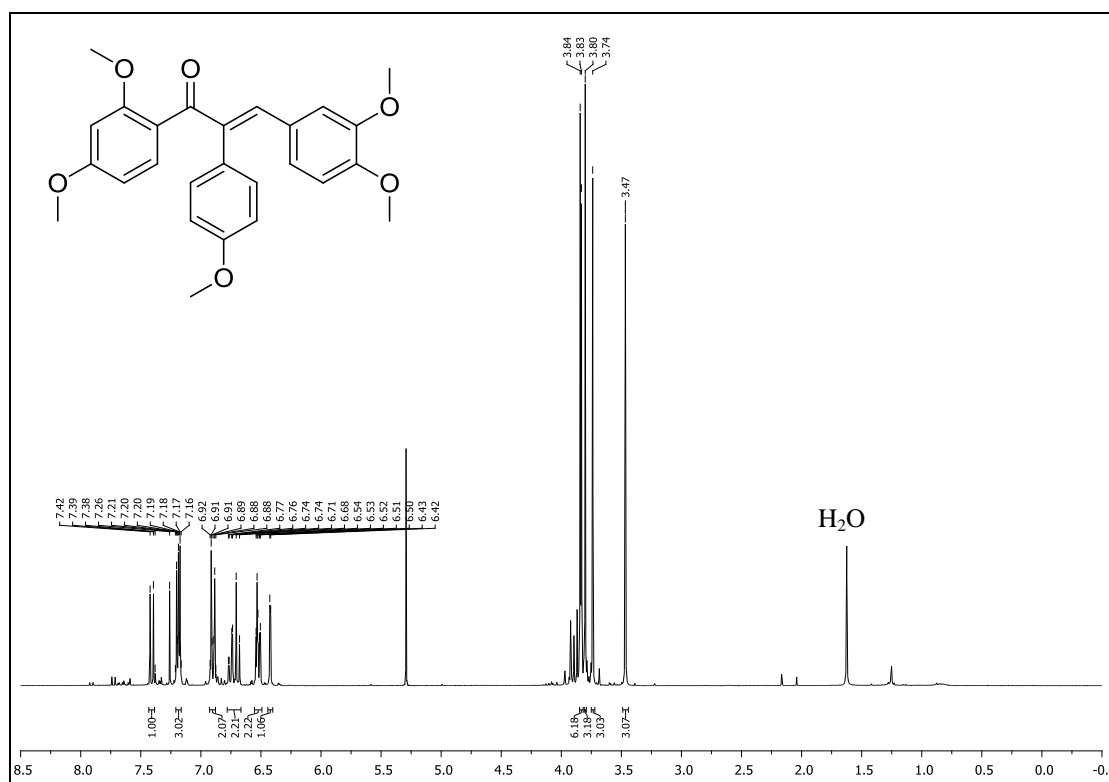
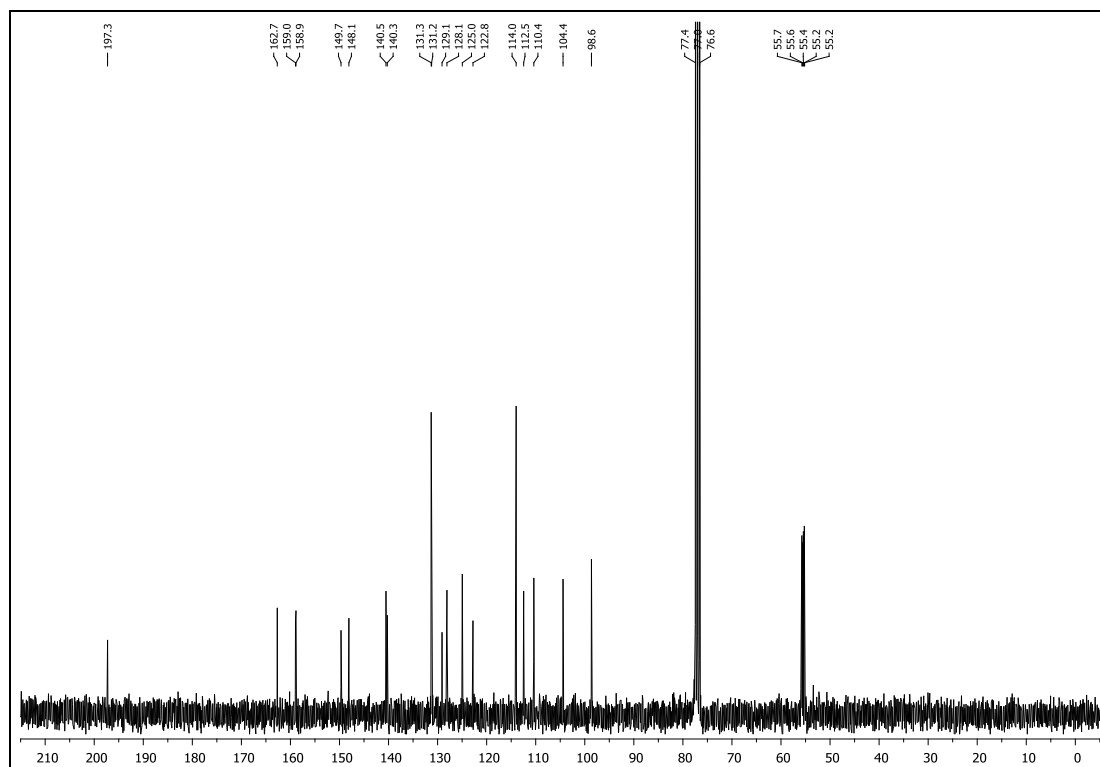


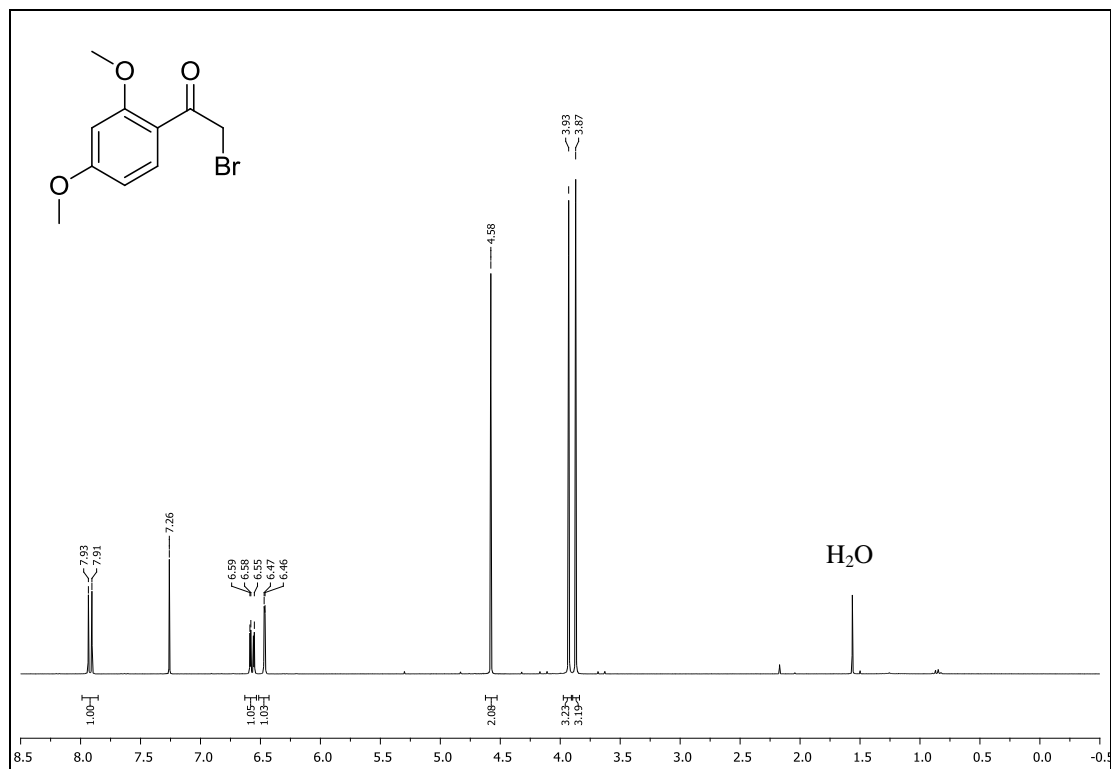
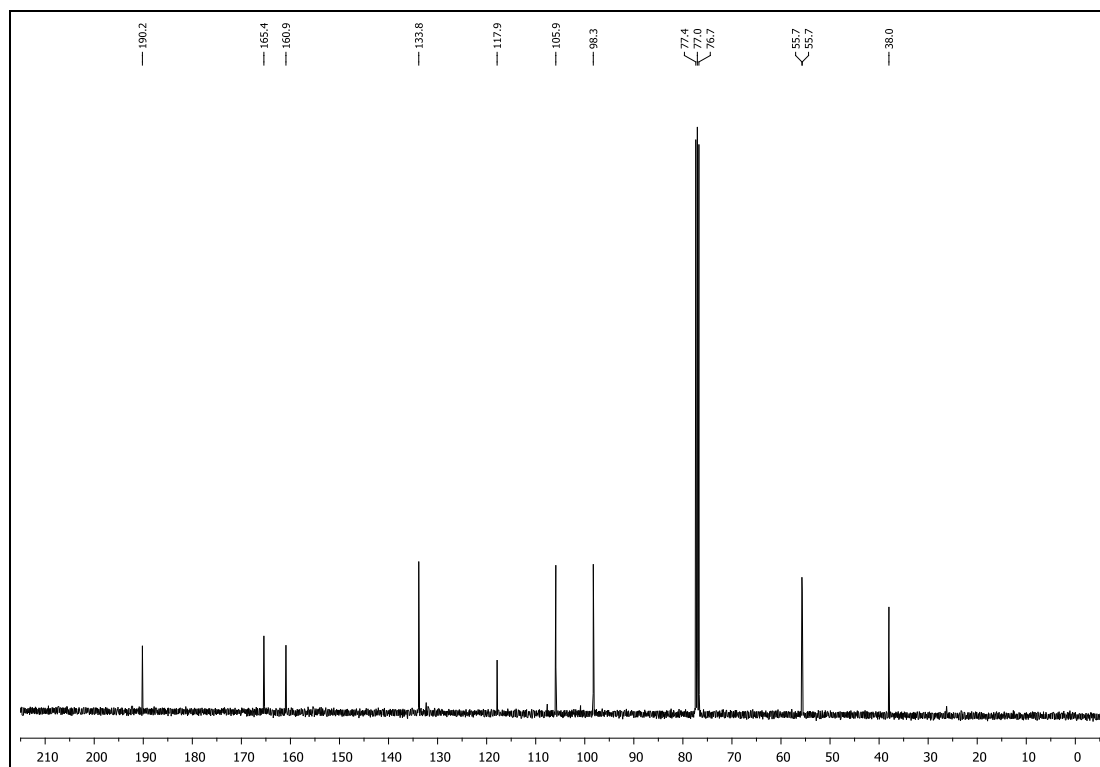
^{19}F NMR

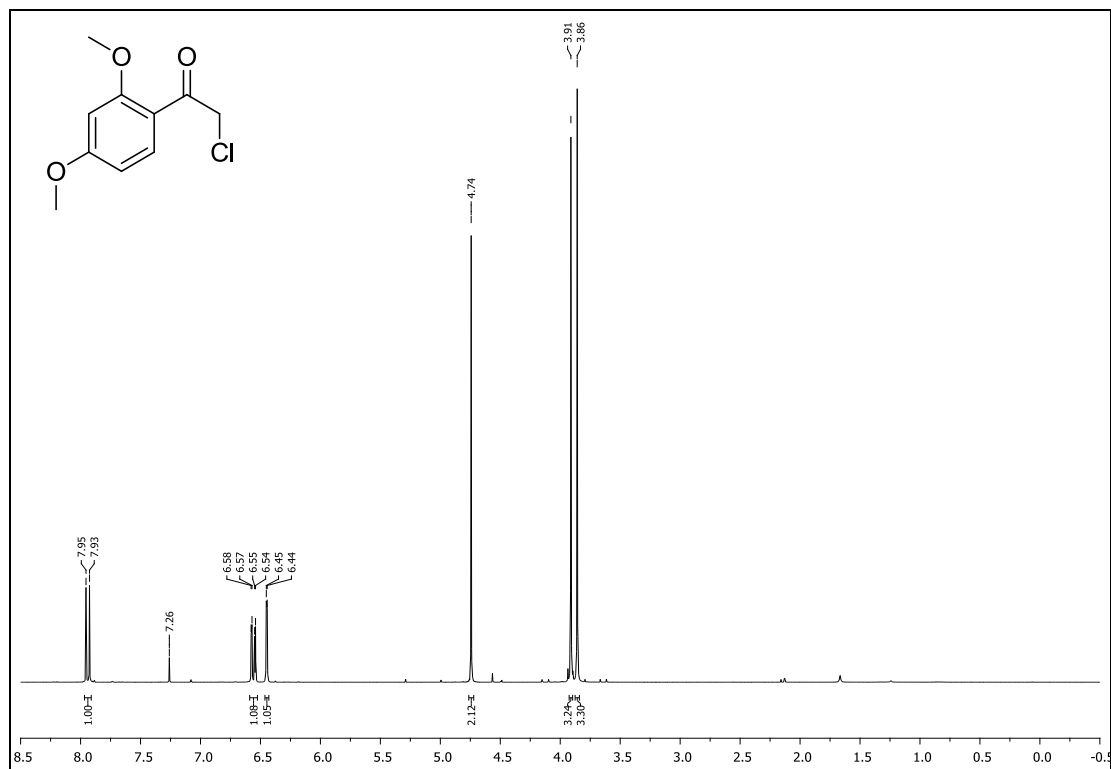
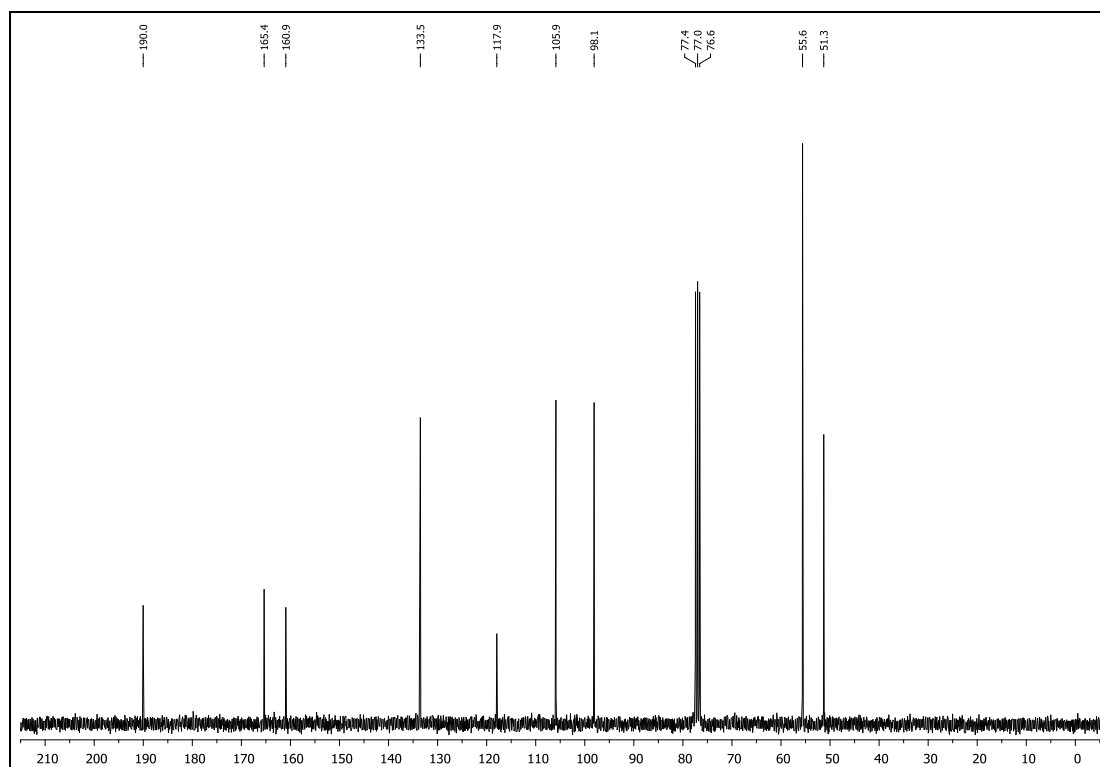
§ Traces of the other isomer

**(E)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-(4-nitrophenyl)prop-2-en-1-one
(78)**¹H NMR¹³C NMR

(E)-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-phenylprop-2-en-1-one (79)¹H NMR¹³C NMR

***E*-1-(2,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-2-(4-methoxyphenyl)prop-2-en-1-one (80)**¹H NMR¹³C NMR

2-Bromo-1-(2,4-dimethoxyphenyl)ethanone (81) ^1H NMR ^{13}C NMR

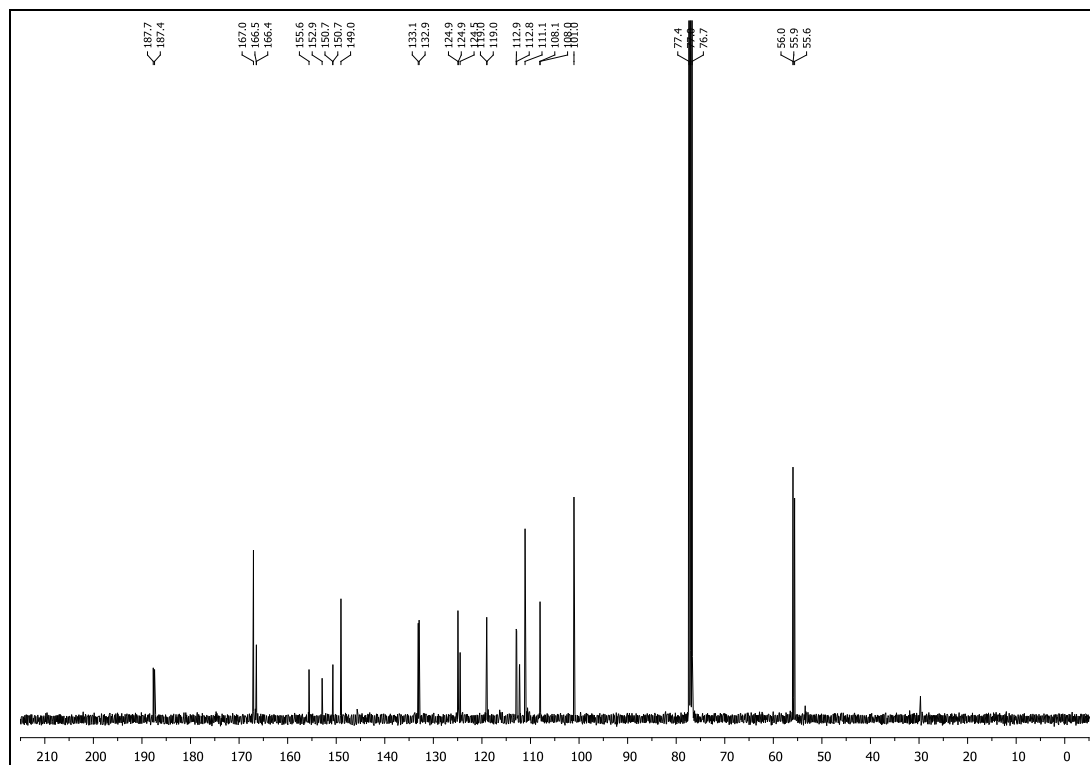
2-Chloro-1-(2,4-dimethoxyphenyl)ethanone (82)¹H NMR¹³C NMR

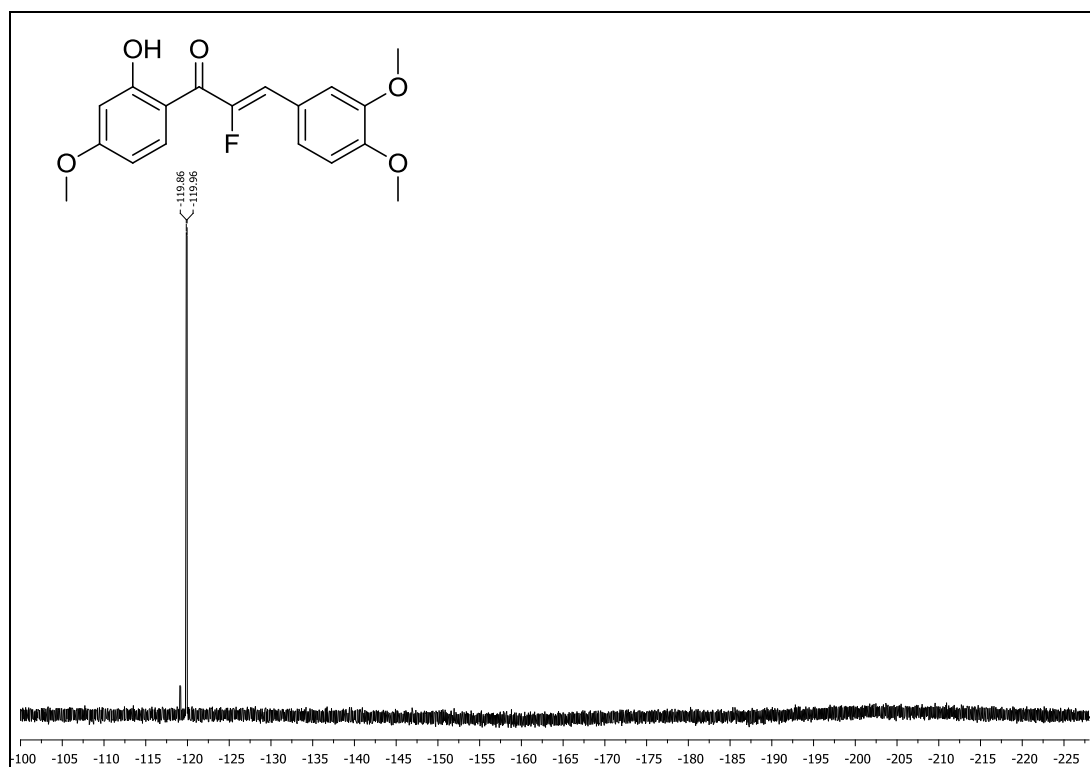
(Z)-3-(3,4-Dimethoxyphenyl)-2-fluoro-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one
(α -F-HC, 94)

^1H NMR



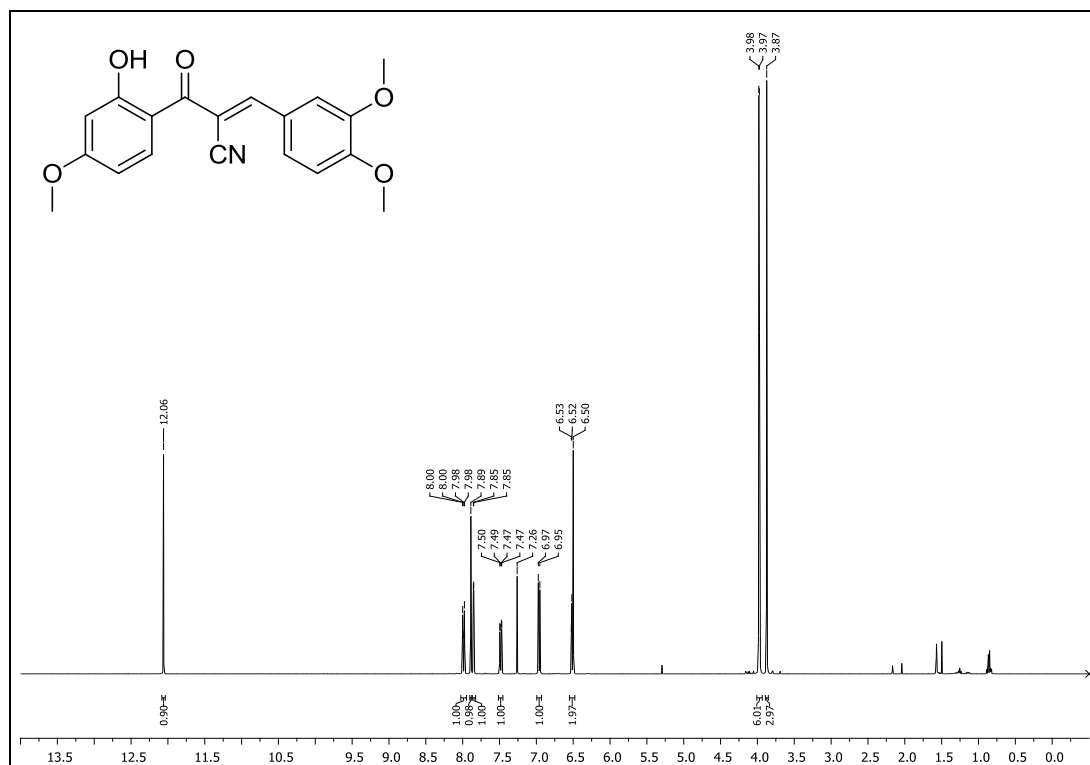
^{13}C NMR



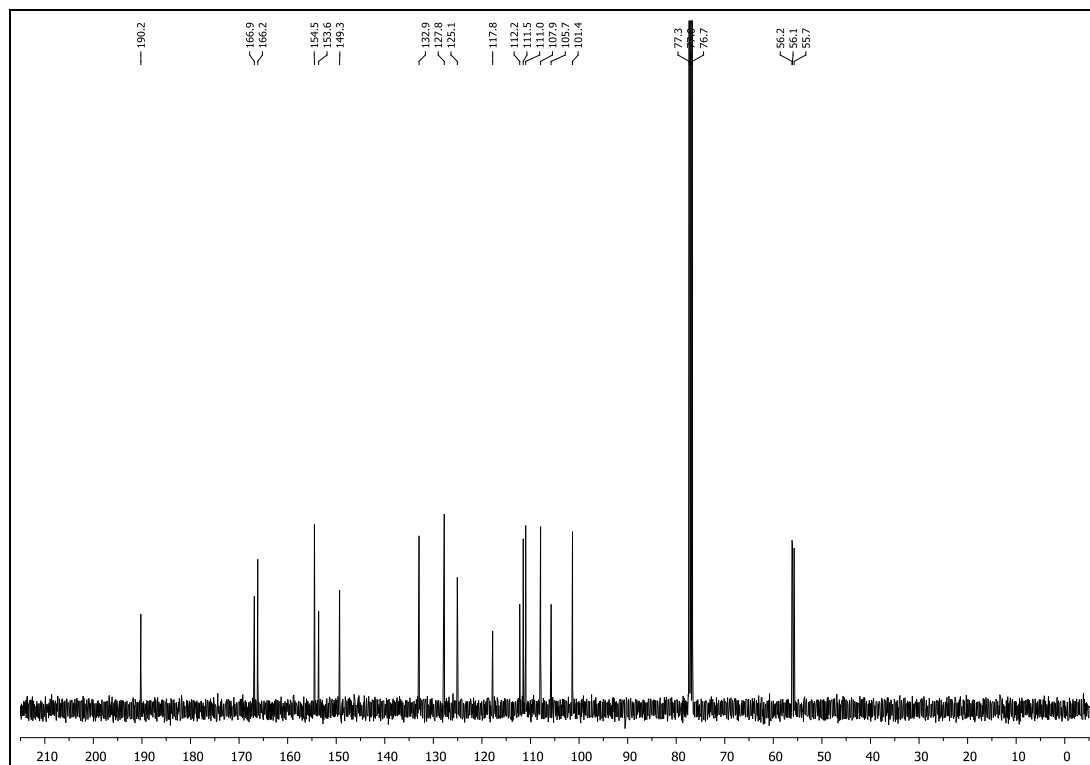
¹⁹F NMR

(E)-2-Cyano-1-(3,4-dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one
(α -CN-HC, 95)

^1H NMR



^{13}C NMR

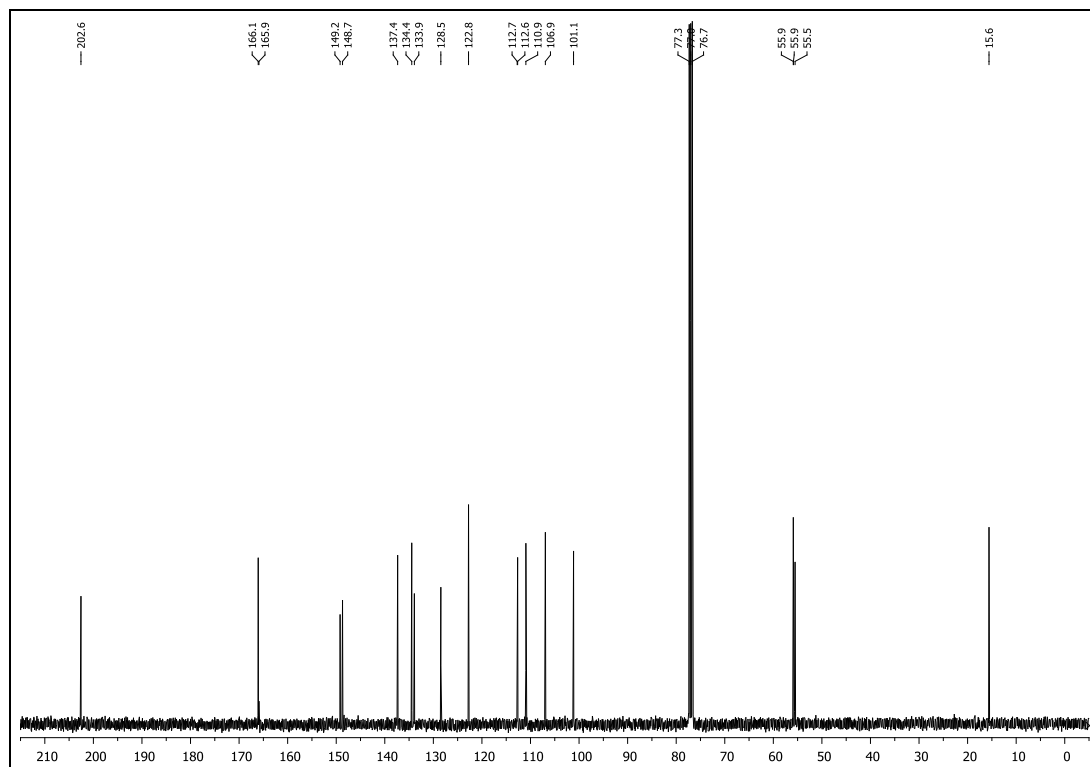


(E)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)-2-methylprop-2-en-1-one
(α -Me-HC, 96)

^1H NMR

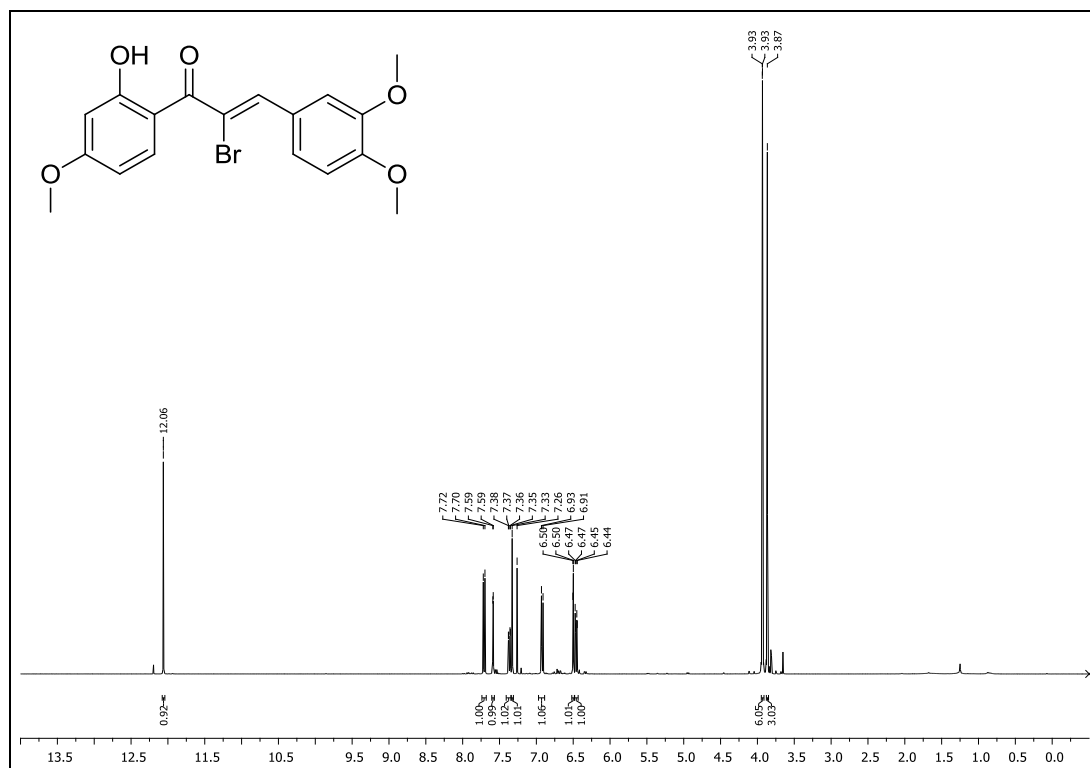


^{13}C NMR

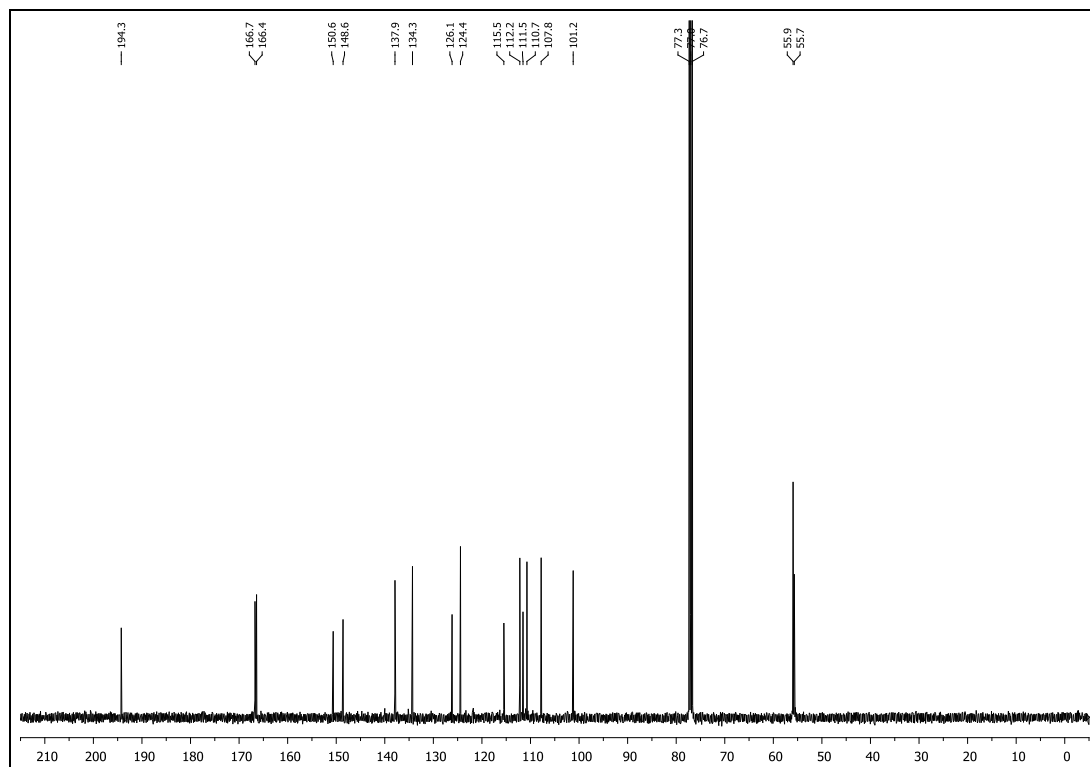


(Z)-2-Bromo-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one
(α -Br-HC, 97)

^1H NMR



^{13}C NMR

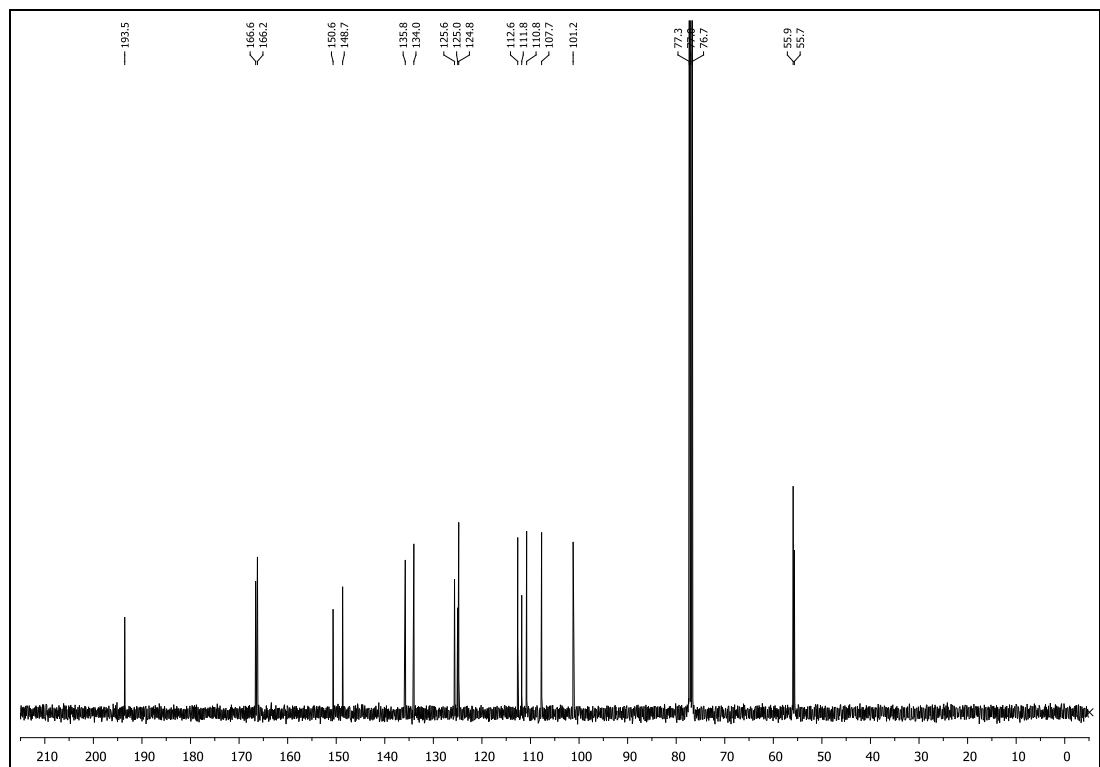


(Z)-2-Chloro-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one
(α -Cl-HC, 98)

^1H NMR

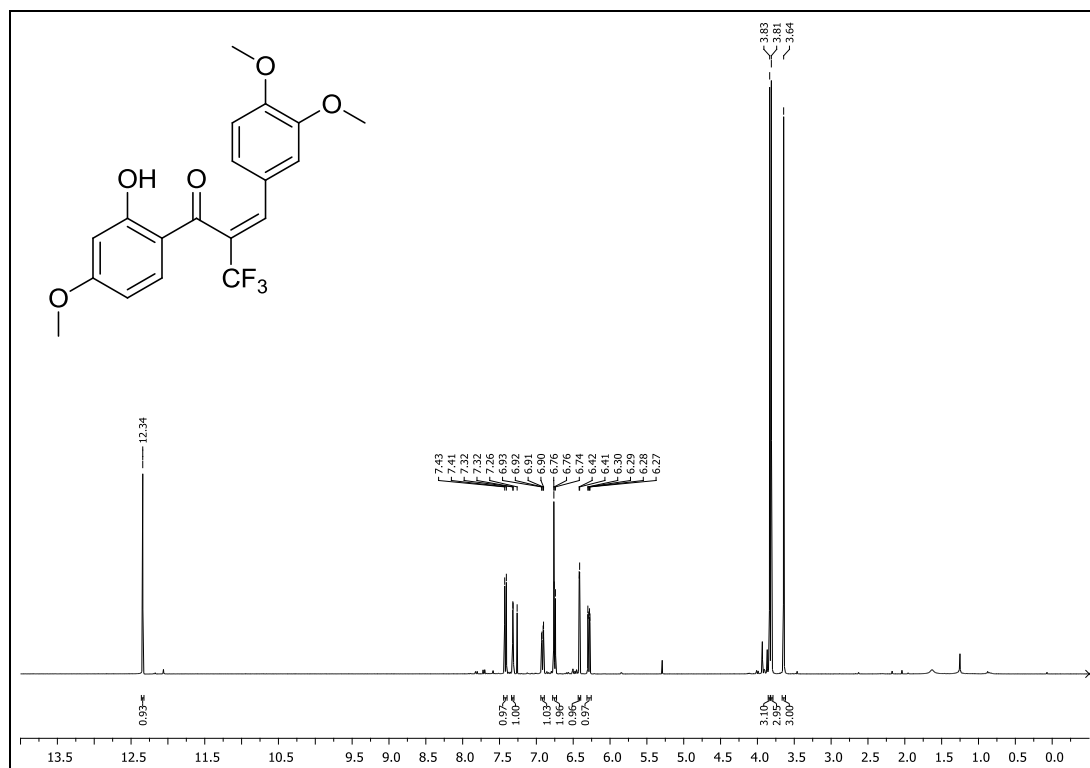


^{13}C NMR

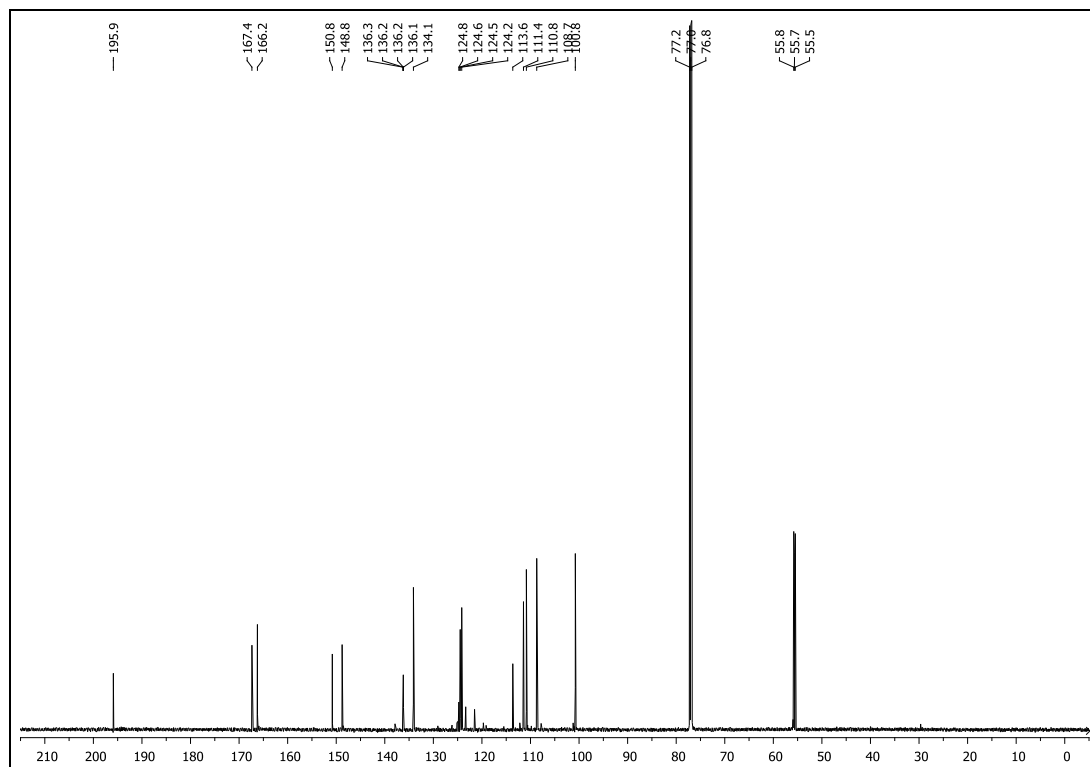


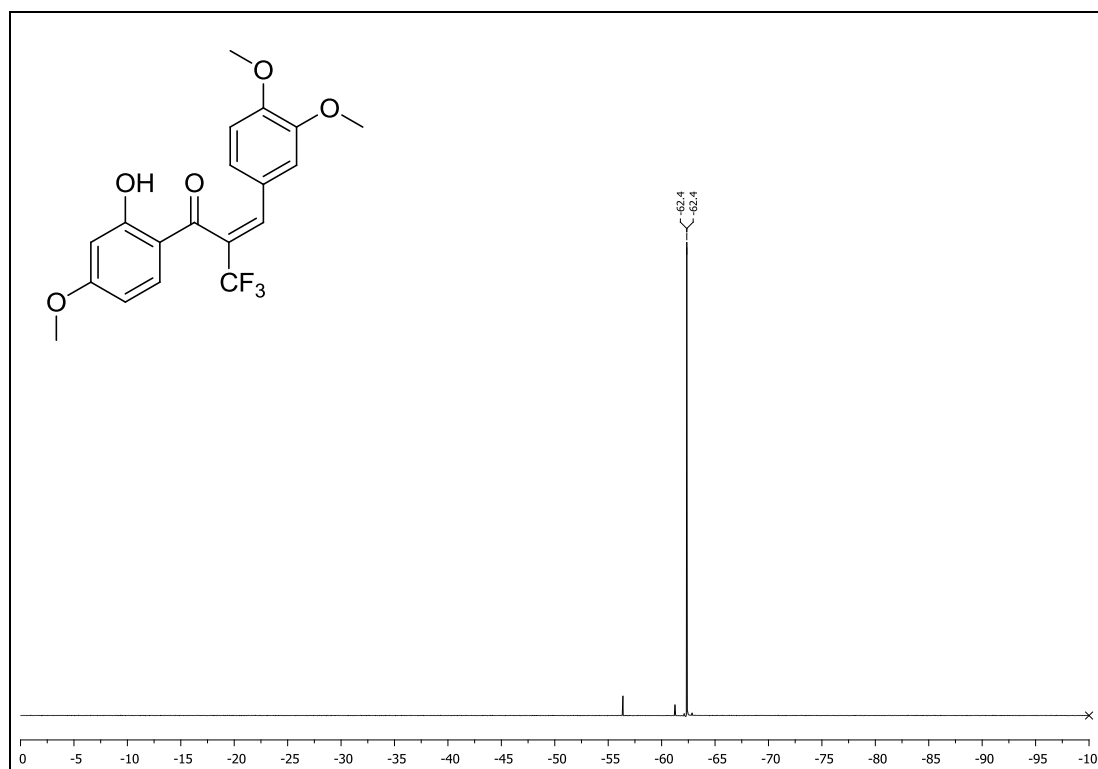
(Z)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)-2-(trifluoromethyl)prop-2-en-1-one (α -CF₃-HC, 99)

¹H NMR



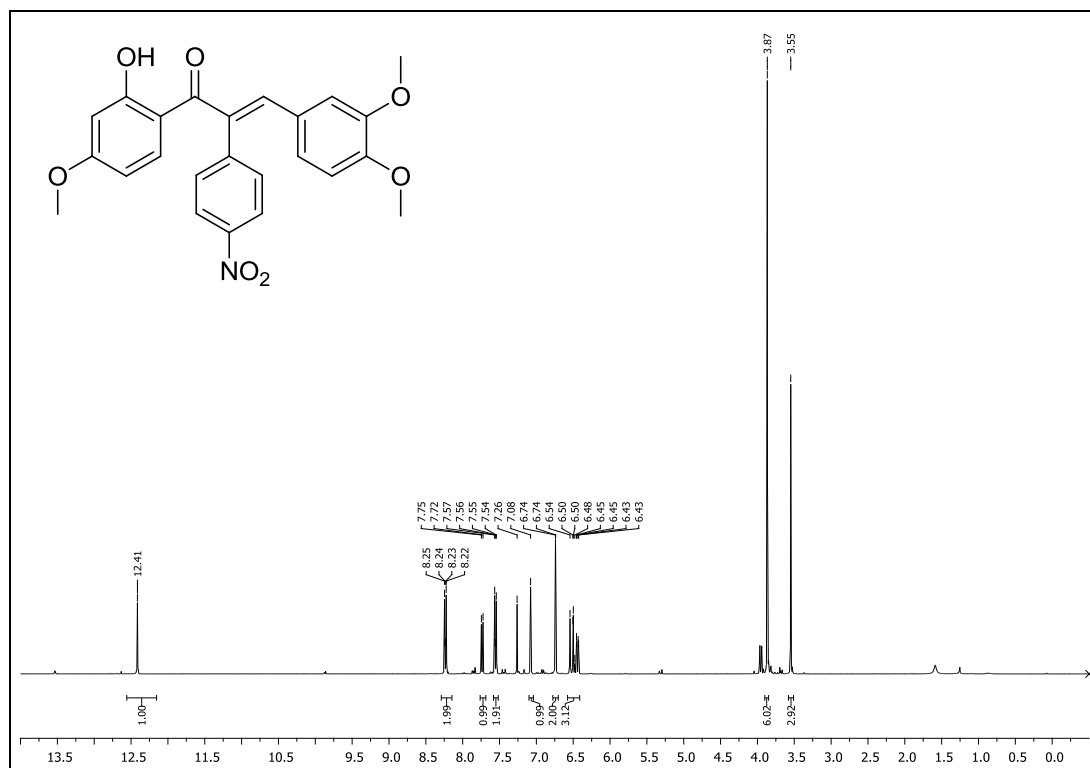
¹³C NMR



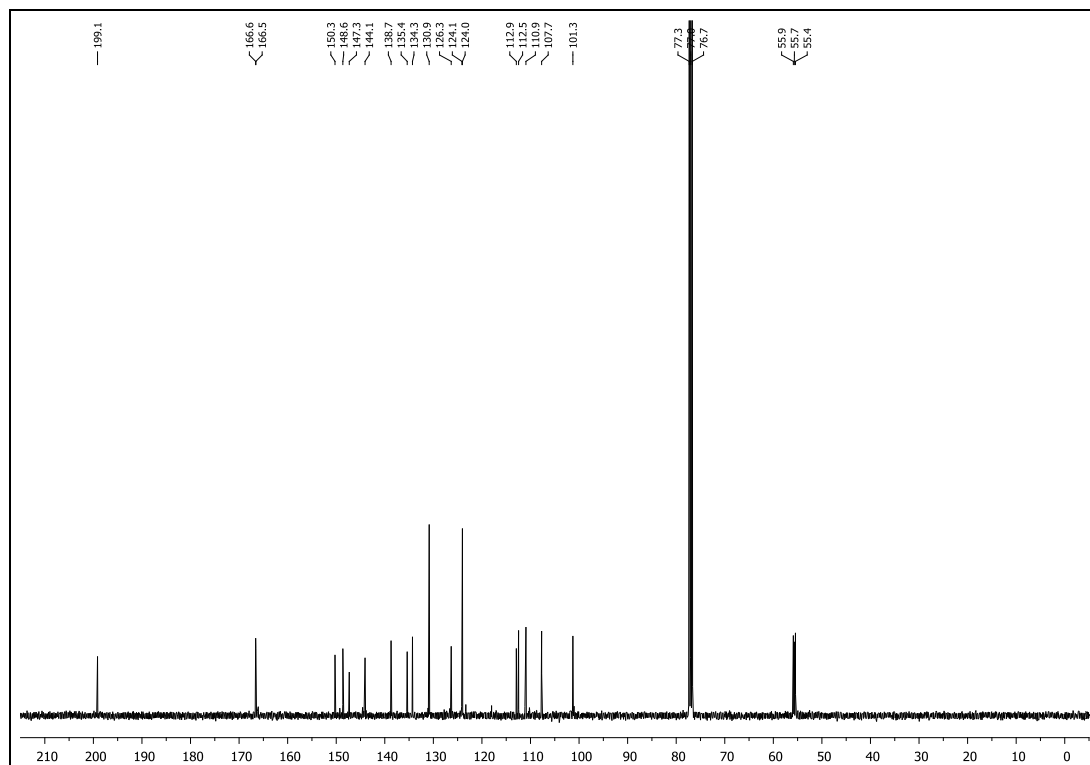
^{19}F NMR

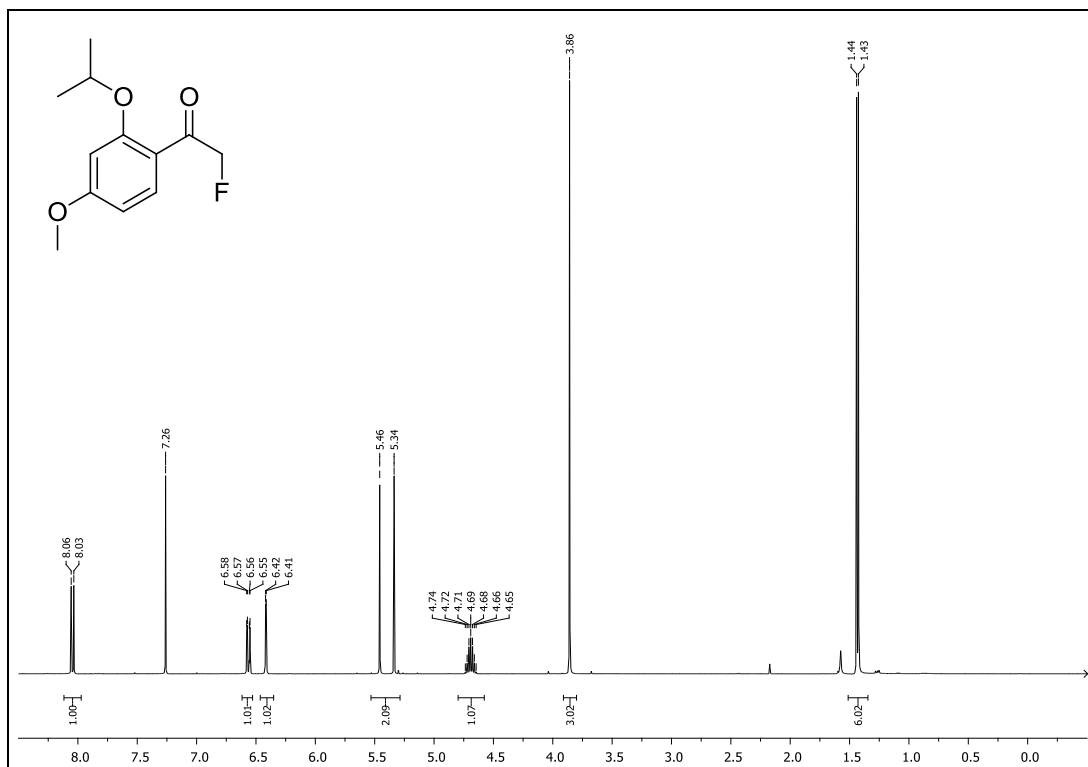
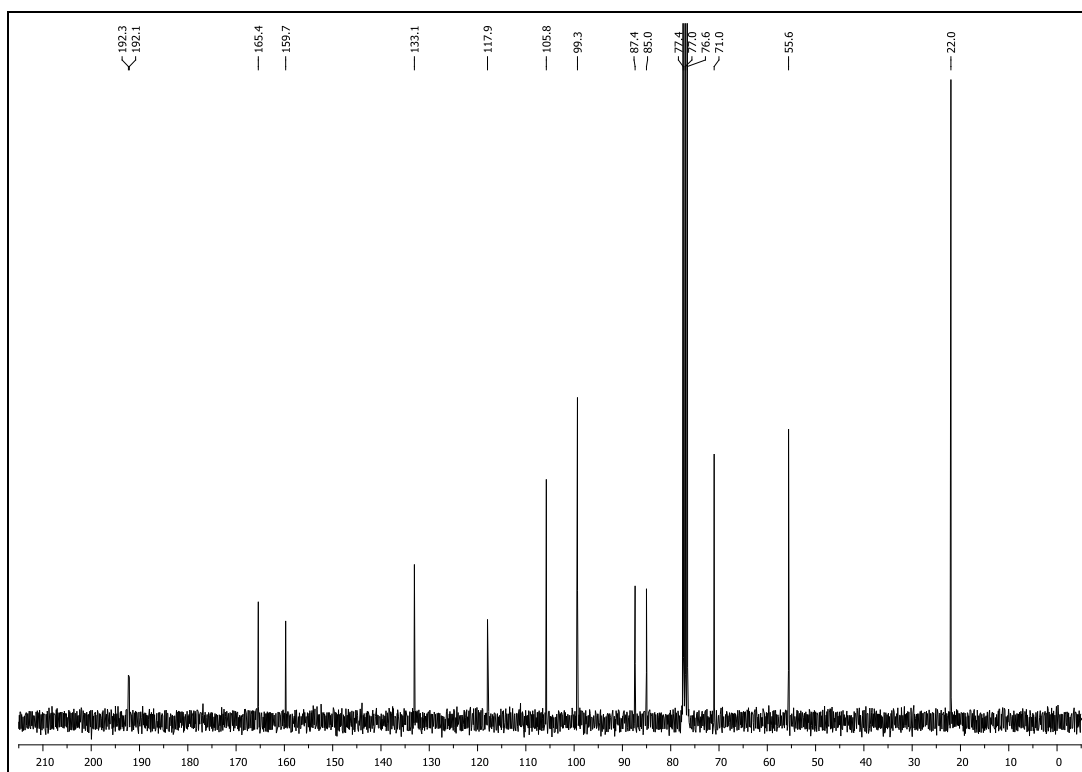
(E)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)-2-(4-nitrophenyl)prop-2-en-1-one (α -p-NO₂-C₆H₄-HC, 100)

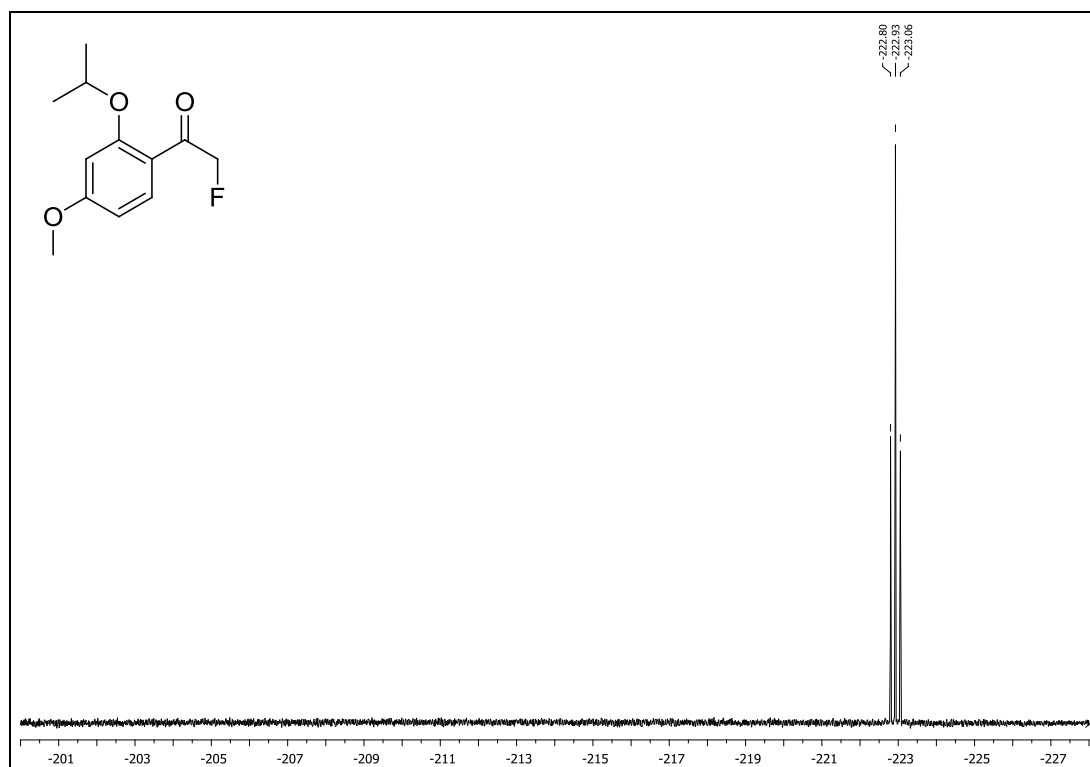
¹H NMR

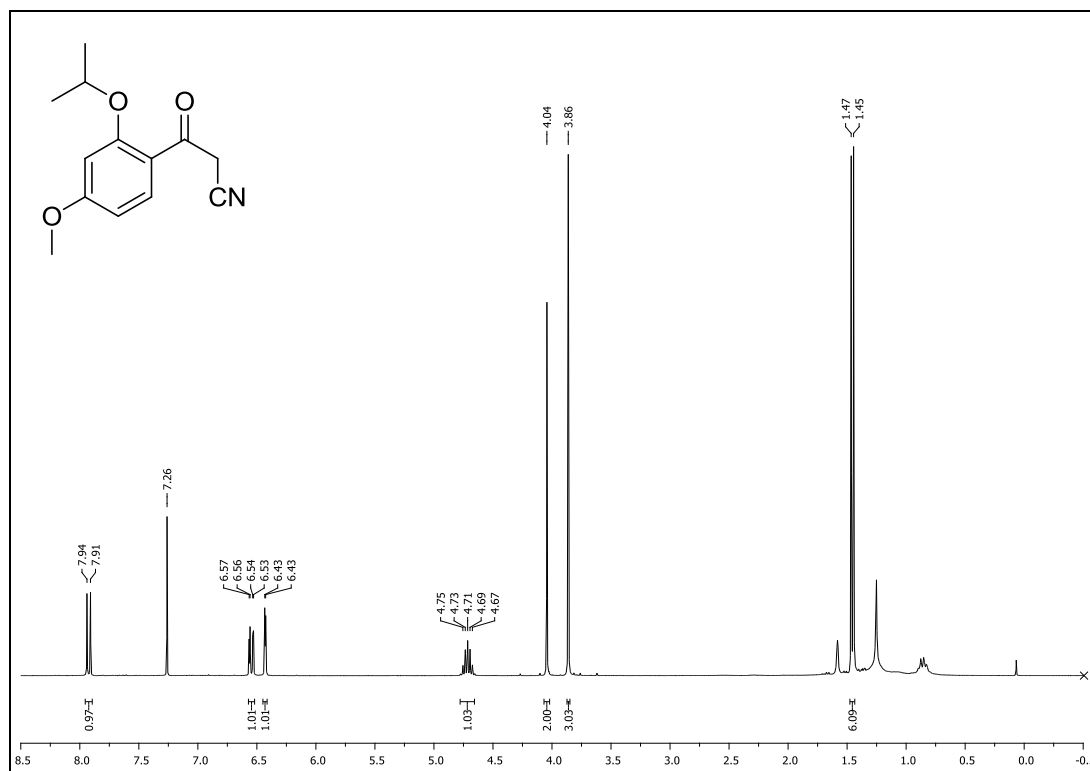
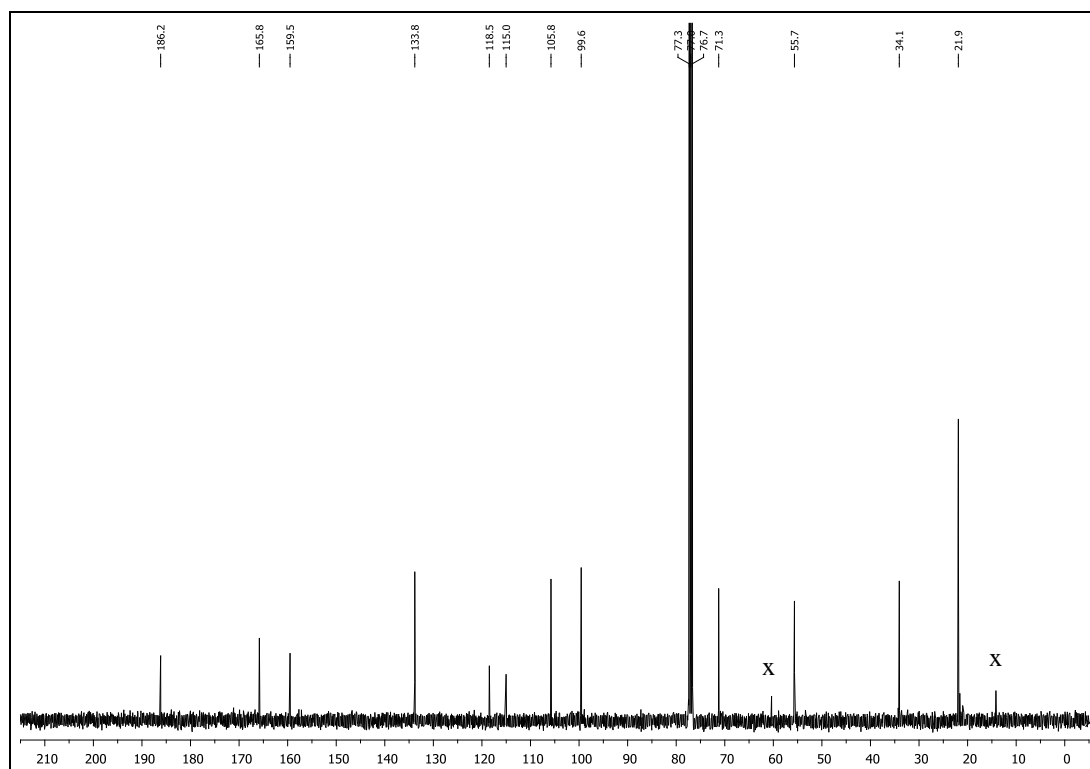


¹³C NMR

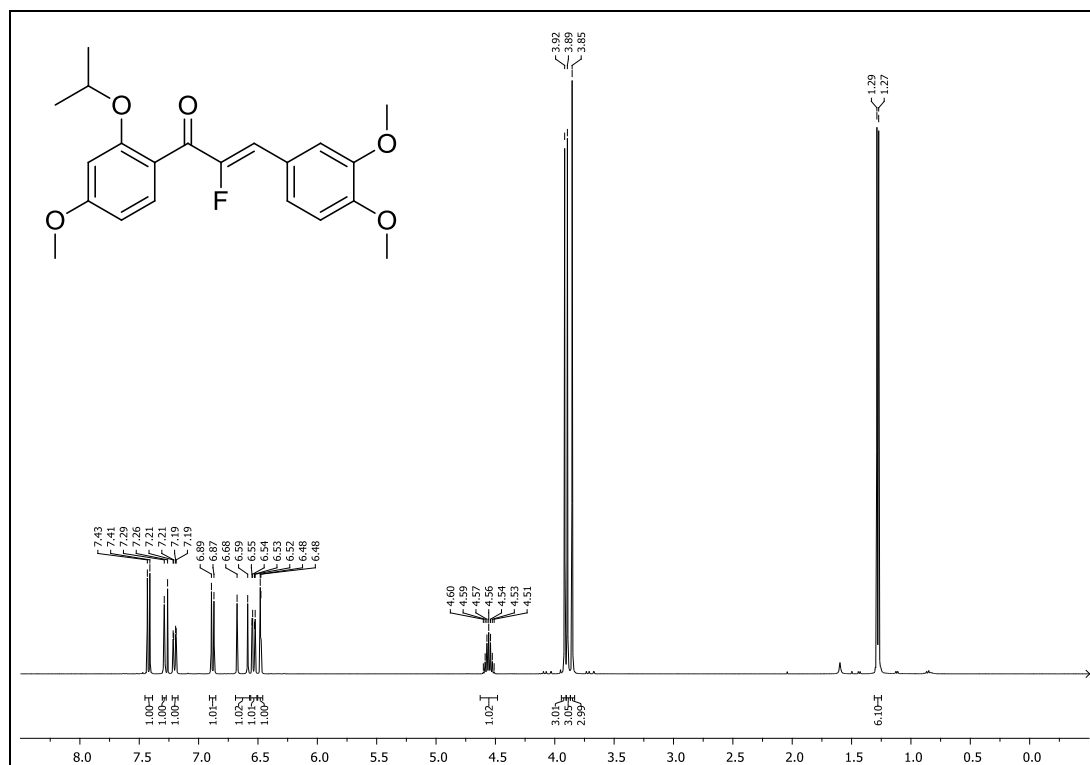
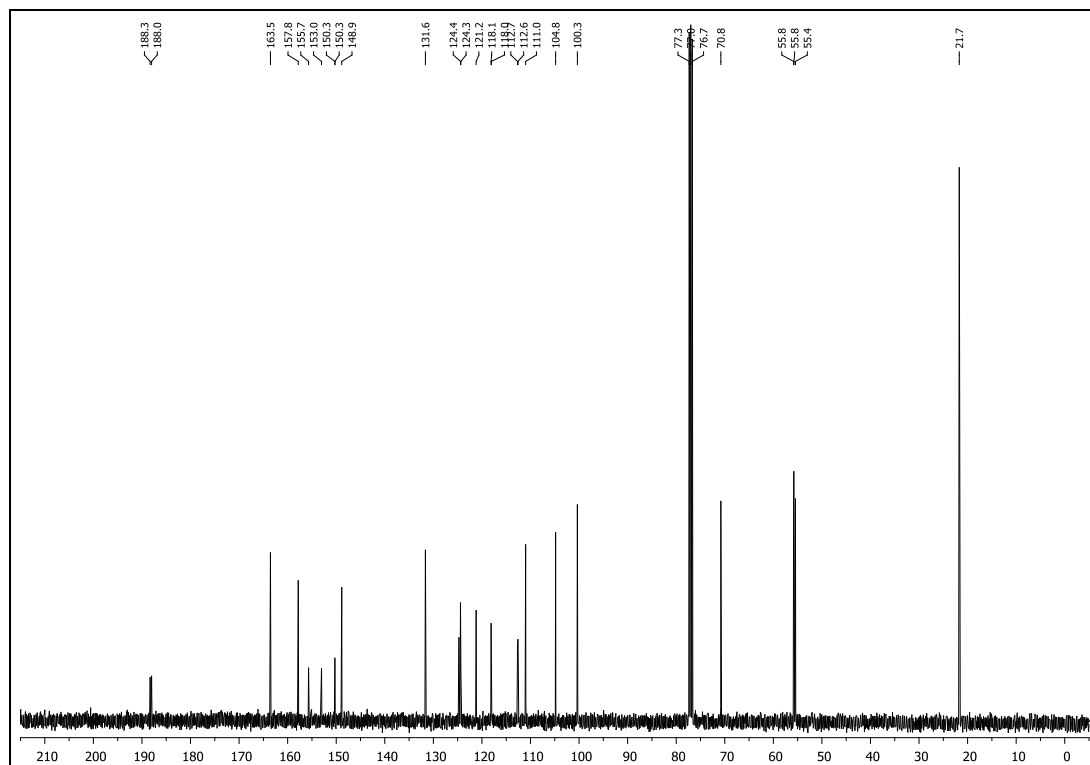


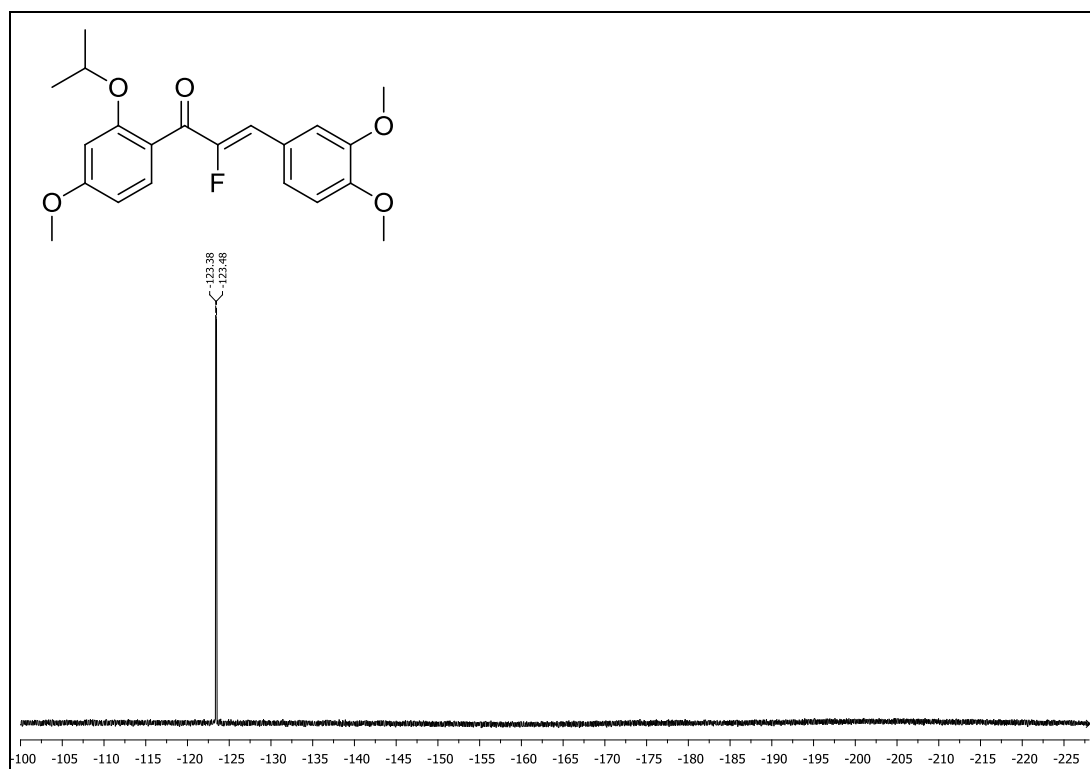
2-Fluoro-1-(2-isopropoxy-4-methoxyphenyl)ethanone (102)¹H NMR¹³C NMR

^{19}F NMR

3-(2-Isopropoxy-4-methoxyphenyl)-3-oxopropanenitrile (103)¹H NMR¹³C NMR

x: impurity

(Z)-3-(3,4-Dimethoxyphenyl)-2-fluoro-1-(2-isopropoxy-4-methoxyphenyl)prop-2-en-1-one (α -F-pHC, 104)¹H NMR¹³C NMR

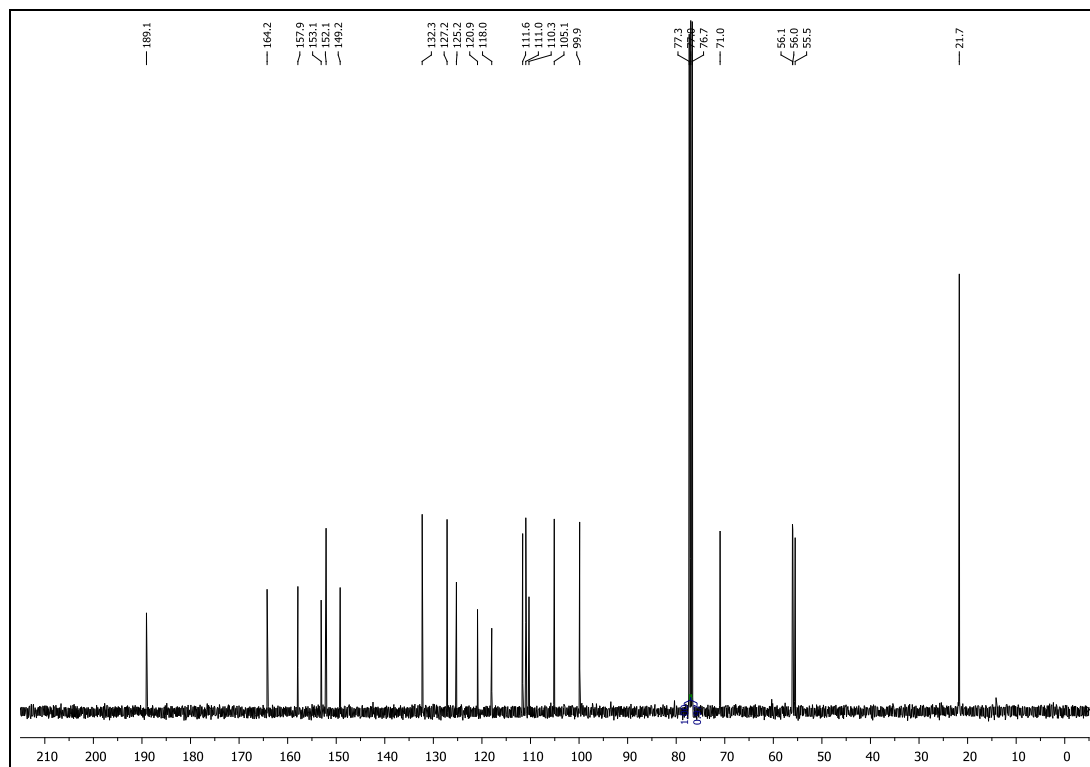
^{19}F NMR

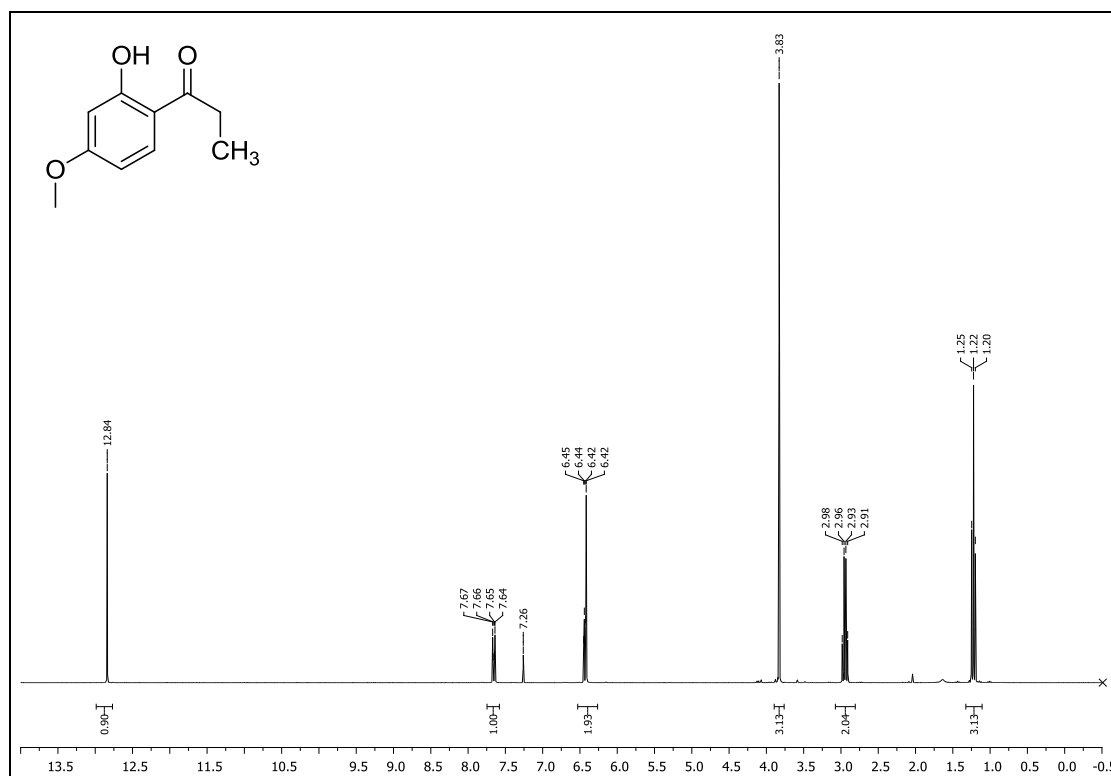
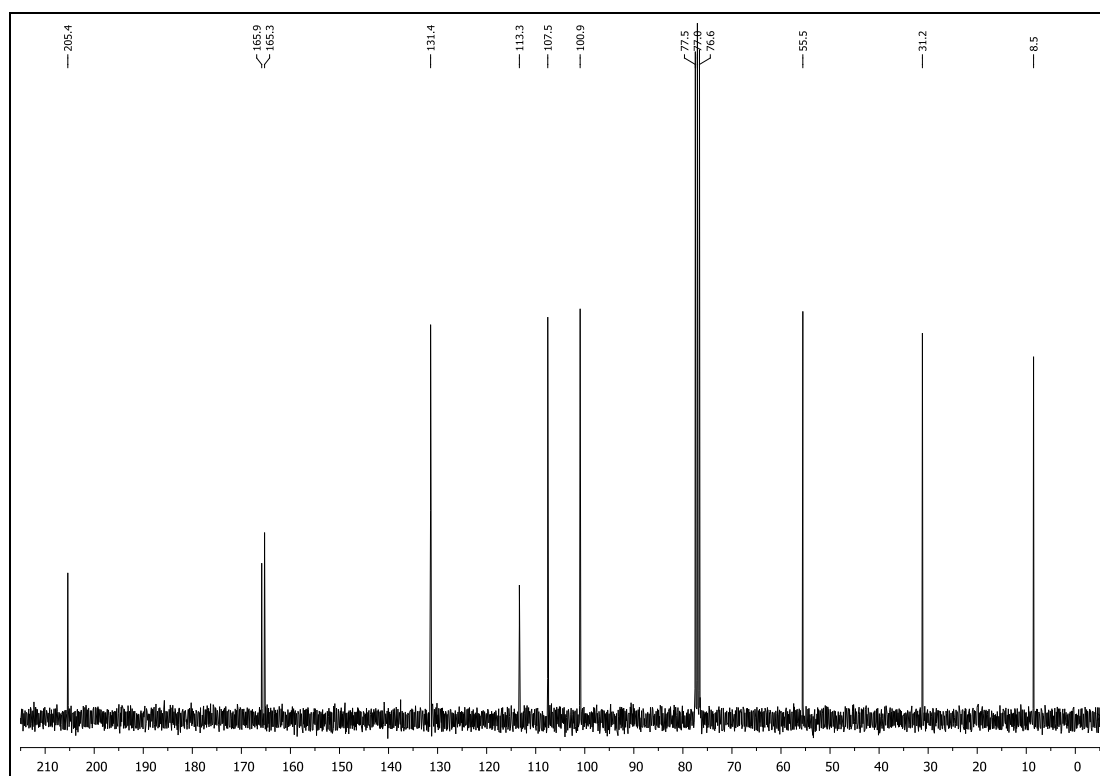
(E)-2-Cyano-3-(3,4-dimethoxyphenyl)-1-(2-isopropoxy-4-methoxyphenyl)prop-2-en-1-one (α -CN-pHC, 105)

^1H NMR



^{13}C NMR



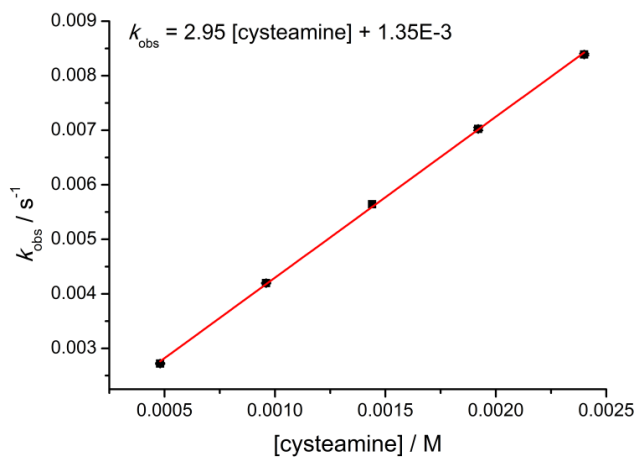
1-(2-Hydroxy-4-methoxyphenyl)propan-1-one (106)¹H NMR¹³C NMR

7.4. Kinetic measurements

7.4.1. Representative k_2 value determinations for α -H-chalcones with cysteamine

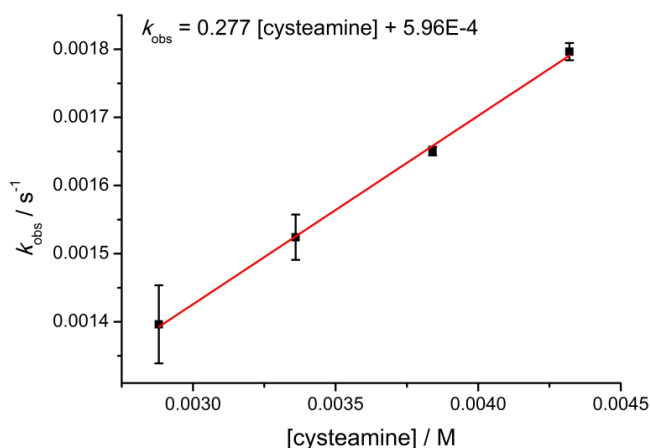
Chalcone (1)

Entry	Thiol [M] (fold)	Individual k_{obs} [s^{-1}]	k_{obs} [s^{-1}] Mean
Abs3	0.00480	2.71×10^{-3}	2.72×10^{-3}
Abs4	(12)	2.73×10^{-3}	
Abs5	0.00960	4.20×10^{-3}	4.20×10^{-3}
Abs6	(24)	4.19×10^{-3}	
Abs7	0.0144	5.64×10^{-3}	5.64×10^{-3}
Abs8	(36)	---	
Abs9	0.0192	7.00×10^{-3}	7.02×10^{-3}
Abs10	(48)	7.05×10^{-3}	
Abs11	0.0240	8.41×10^{-3}	8.39×10^{-3}
Abs12	(60)	8.37×10^{-3}	
$k_2 = 2.95 \text{ M}^{-1} \text{ s}^{-1}$			



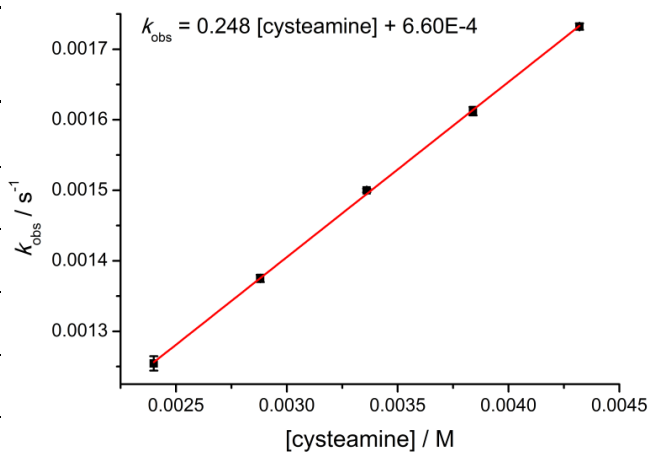
Butein (2)

Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean
Abs3	0.00240	---	---
Abs4	(60)	---	
Abs5	0.00288	1.45×10^{-3}	1.40×10^{-3}
Abs6	(72)	1.34×10^{-3}	
Abs7	0.00336	1.56×10^{-3}	1.52×10^{-3}
Abs8	(84)	1.49×10^{-3}	
Abs9	0.00384	1.66×10^{-3}	1.65×10^{-3}
Abs10	(96)	1.64×10^{-3}	
Abs11	0.00432	1.81×10^{-3}	1.80×10^{-3}
Abs12	(108)	1.78×10^{-3}	
$k_2 = 0.277 \text{ M}^{-1} \text{ s}^{-1}$			



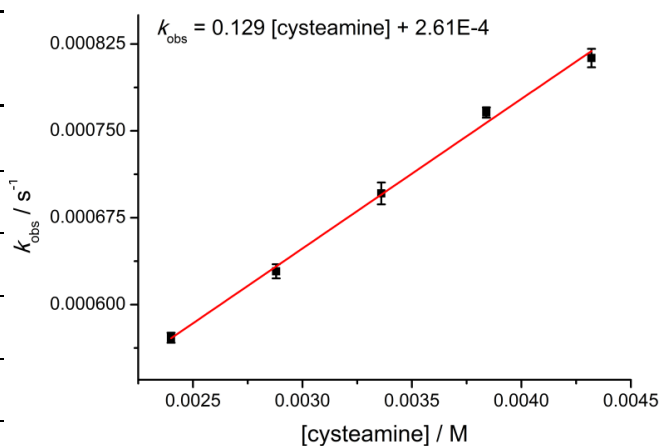
Isoliquiritigenin (4)

Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean
Abs3	0.00240	1.24×10^{-3}	1.25×10^{-3}
Abs4	(60)	1.26×10^{-3}	
Abs5	0.00288	1.38×10^{-3}	1.38×10^{-3}
Abs6	(72)	1.37×10^{-3}	
Abs7	0.00336	1.50×10^{-3}	1.50×10^{-3}
Abs8	(84)	1.50×10^{-3}	
Abs9	0.00384	1.61×10^{-3}	1.61×10^{-3}
Abs10	(96)	1.62×10^{-3}	
Abs11	0.00432	1.73×10^{-3}	1.73×10^{-3}
Abs12	(108)	1.74×10^{-3}	
$k_2 = 0.248 \text{ M}^{-1} \text{ s}^{-1}$			

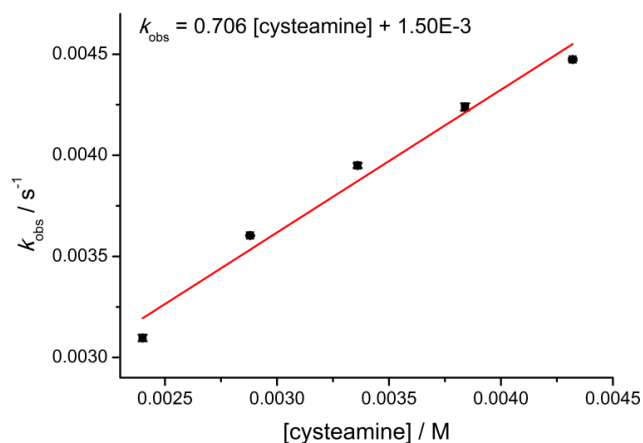


Xanthohumol (**5**)

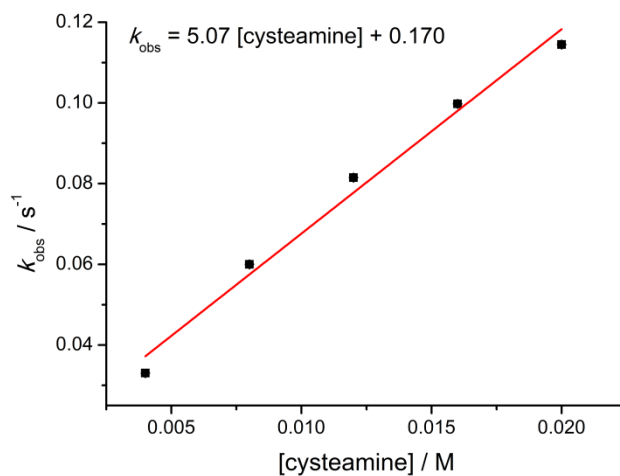
Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean
Abs3	0.00240	5.76×10^{-4}	5.71×10^{-4}
Abs4	(60)	5.67×10^{-4}	
Abs5	0.00288	6.35×10^{-4}	6.29×10^{-4}
Abs6	(72)	6.23×10^{-4}	
Abs7	0.00336	7.05×10^{-4}	6.96×10^{-4}
Abs8	(84)	6.87×10^{-4}	
Abs9	0.00384	7.70×10^{-4}	7.66×10^{-4}
Abs10	(96)	7.61×10^{-4}	
Abs11	0.00432	8.21×10^{-4}	8.13×10^{-4}
Abs12	(108)	8.05×10^{-4}	
$k_2 = 0.129 \text{ M}^{-1} \text{ s}^{-1}$			

*(E)*-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxy-phenyl)prop-2-en-1-one (α -H-HC, **61**)

Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean
Abs3	0.00240	3.08×10^{-3}	3.10×10^{-3}
Abs4	(60)	3.11×10^{-3}	
Abs5	0.00288	3.61×10^{-3}	3.60×10^{-3}
Abs6	(72)	3.60×10^{-3}	
Abs7	0.00336	3.94×10^{-3}	3.95×10^{-3}
Abs8	(84)	3.96×10^{-3}	
Abs9	0.00384	4.26×10^{-3}	4.24×10^{-3}
Abs10	(96)	4.22×10^{-3}	
Abs11	0.00432	4.47×10^{-3}	4.47×10^{-3}
Abs12	(108)	4.48×10^{-3}	
$k_2 = 0.706 \text{ M}^{-1} \text{ s}^{-1}$			

*(E)*-1-(2-Hydroxyphenyl)-3-phenylprop-2-en-1-one (**62**)

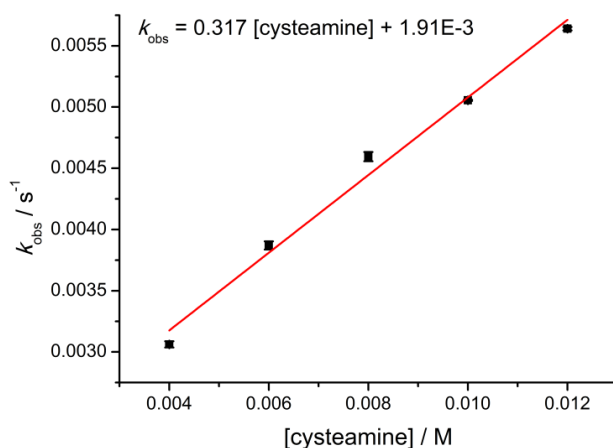
Entry	Thiol [M] (fold)	k_{obs} [s^{-1}] Mean of replicates
1	0.00400 (100)	0.0331
2	0.00800 (200)	0.0600
3	0.0120 (300)	0.0815
4	0.0160 (400)	0.100
5	0.0200 (500)	0.114
$k_2 = 5.07 \text{ M}^{-1} \text{ s}^{-1}$		



Calythropsin (**116**)

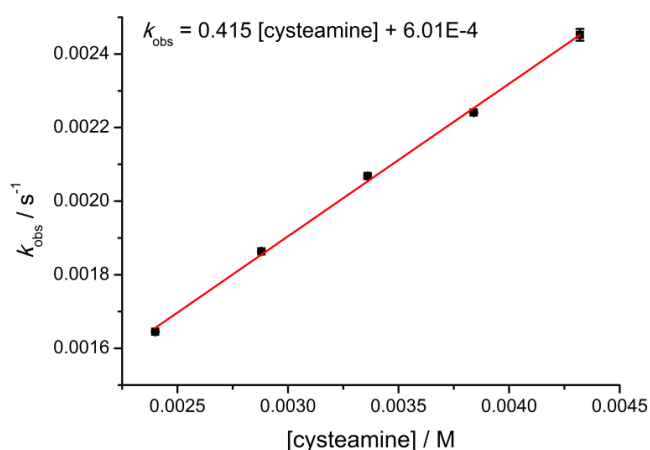
Entry	Thiol [M] (fold)	Individual k_{obs} [s^{-1}]	k_{obs} [s^{-1}] Mean
Abs3	0.00400	3.09×10^{-3}	3.06×10^{-3}
Abs4	(100)	3.04×10^{-3}	
Abs5	0.00600	3.90×10^{-3}	3.87×10^{-3}
Abs6	(150)	3.84×10^{-3}	
Abs7	0.00800	4.63×10^{-3}	4.60×10^{-3}
Abs8	(200)	4.56×10^{-3}	
Abs9	0.0100	5.06×10^{-3}	5.05×10^{-3}
Abs10	(250)	5.05×10^{-3}	
Abs11	0.0120	5.65×10^{-3}	5.64×10^{-3}
Abs12	(300)	5.63×10^{-3}	

$$k_2 = 0.317 \text{ M}^{-1} \text{ s}^{-1}$$

4-Methoxy-2',3,4'-trihydroxychalcone (**118**)

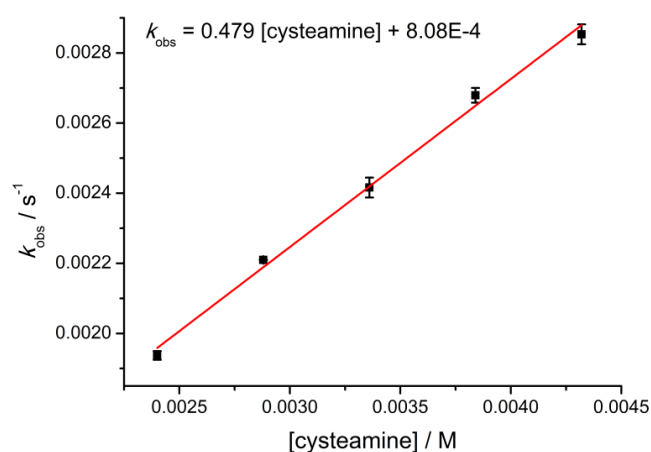
Entry	Thiol [M] (fold)	Individual k_{obs} [s^{-1}]	k_{obs} [s^{-1}] Mean
Abs3	0.00240	1.65×10^{-3}	1.65×10^{-3}
Abs4	(60)	1.64×10^{-3}	
Abs5	0.00288	1.87×10^{-3}	1.86×10^{-3}
Abs6	(72)	1.86×10^{-3}	
Abs7	0.00336	2.06×10^{-3}	2.07×10^{-3}
Abs8	(84)	2.07×10^{-3}	
Abs9	0.00384	2.24×10^{-3}	2.24×10^{-3}
Abs10	(96)	2.24×10^{-3}	
Abs11	0.00432	2.47×10^{-3}	2.45×10^{-3}
Abs12	(108)	2.44×10^{-3}	

$$k_2 = 0.415 \text{ M}^{-1} \text{ s}^{-1}$$

2',4'-Dihydroxy-3,4-dimethoxychalcone (**119**)

Entry	Thiol [M] (fold)	Individual k_{obs} [s^{-1}]	k_{obs} [s^{-1}] Mean
Abs3	0.00240	1.92×10^{-3}	1.94×10^{-3}
Abs4	(60)	1.95×10^{-3}	
Abs5	0.00288	2.21×10^{-3}	2.21×10^{-3}
Abs6	(72)	2.21×10^{-3}	
Abs7	0.00336	2.39×10^{-3}	2.42×10^{-3}
Abs8	(84)	2.44×10^{-3}	
Abs9	0.00384	2.70×10^{-3}	2.68×10^{-3}
Abs10	(96)	2.66×10^{-3}	
Abs11	0.00432	2.88×10^{-3}	2.85×10^{-3}
Abs12	(108)	2.82×10^{-3}	

$$k_2 = 0.479 \text{ M}^{-1} \text{ s}^{-1}$$



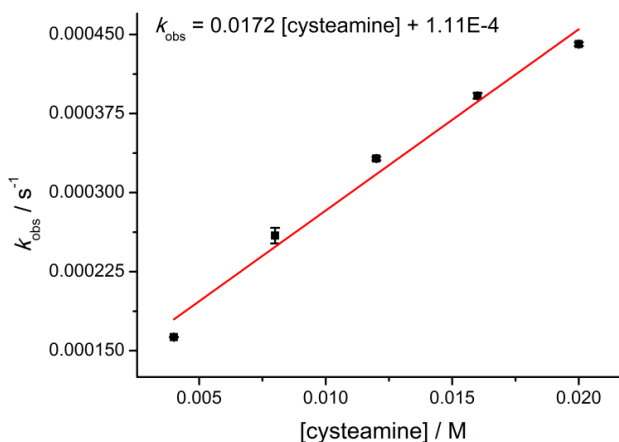
The other α -H-chalcones shown in Table 5 in Results and Discussion part were tested by Katrin Winter.¹¹⁶

7.4.2. Representative k_2 value determinations for α -X-TMCs with cysteamine

α -F-TMC (68)

Entry	Thiol [M] (fold)	Individual k_{obs} [s^{-1}]	k_{obs} [s^{-1}] Mean
Abs3	0.00400	1.63×10^{-4}	1.63×10^{-4}
Abs4	(100)	1.63×10^{-4}	
Abs5	0.00800	2.67×10^{-4}	2.59×10^{-4}
Abs6	(200)	2.52×10^{-4}	
Abs7	0.0120	3.34×10^{-4}	3.32×10^{-4}
Abs8	(300)	3.31×10^{-4}	
Abs9	0.0160	3.89×10^{-4}	3.92×10^{-4}
Abs10	(400)	3.94×10^{-4}	
Abs11	0.0200	4.39×10^{-4}	4.41×10^{-4}
Abs12	(500)	4.43×10^{-4}	

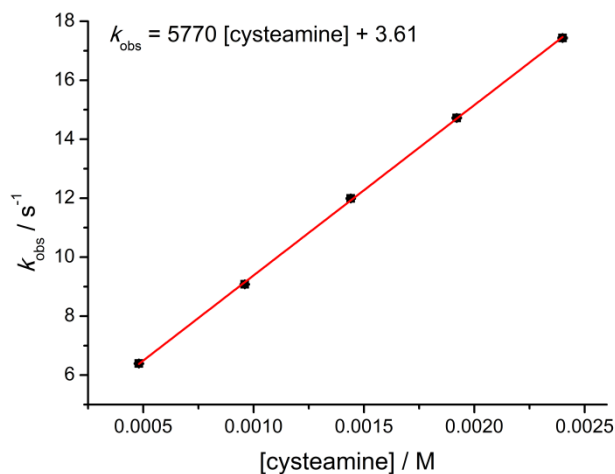
$$k_2 = 0.0172 \text{ M}^{-1} \text{ s}^{-1}$$



α -CN-TMC (69)

Entry	Thiol [M] (fold)	k_{obs} [s^{-1}] Mean of replicates
1	0.00480 (12)	6.39
2	0.00960 (24)	9.08
3	0.0144 (36)	12.0
4	0.0192 (48)	14.7
5	0.0240 (60)	17.4

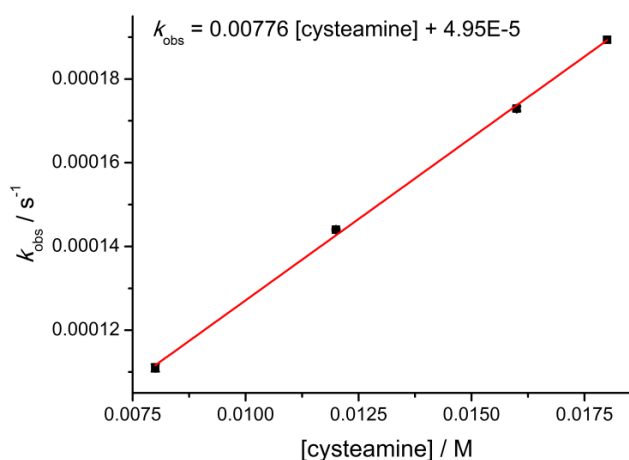
$$k_2 = 5770 \text{ M}^{-1} \text{ s}^{-1}$$



α -Me-TMC (70)

Entry	Thiol [M] (fold)	Individual k_{obs} [s^{-1}]	k_{obs} [s^{-1}] Mean
Abs3	0.00800	1.12×10^{-4}	1.11×10^{-4}
Abs4	(200)	1.10×10^{-4}	
Abs5	0.0120	1.44×10^{-4}	1.44×10^{-4}
Abs6	(300)	1.44×10^{-4}	
Abs7	0.0160	1.74×10^{-4}	1.73×10^{-4}
Abs8	(400)	1.72×10^{-4}	
Abs9	0.0180	1.89×10^{-4}	1.89×10^{-4}
Abs10	(450)	---	
Abs11	0.0200	---	---
Abs12	(500)	---	

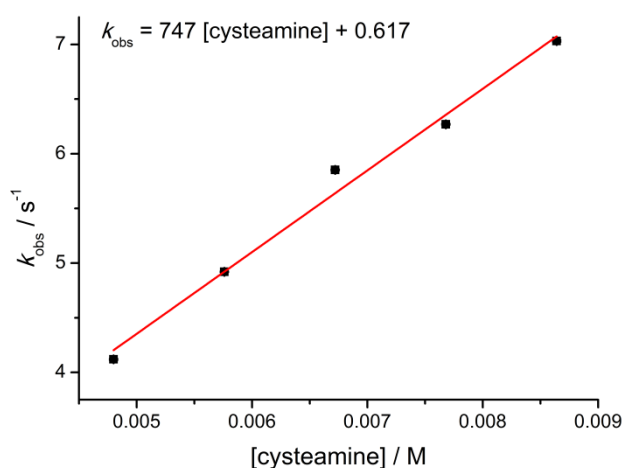
$$k_2 = 0.00776 \text{ M}^{-1} \text{ s}^{-1}$$



α -NO₂-TMC (71)

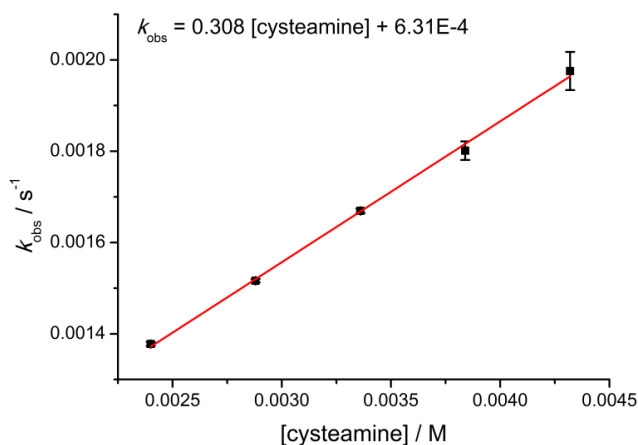
Entry	Thiol [M] (fold)	k_{obs} [s ⁻¹] Mean of replicates
1	0.00480 (60)	4.12
2	0.00576 (72)	4.92
3	0.00672 (84)	5.85
4	0.00768 (96)	6.27
5	0.00864 (108)	7.03

$k_2 = 747 \text{ M}^{-1} \text{ s}^{-1}$

 α -COOEt-TMC (72)

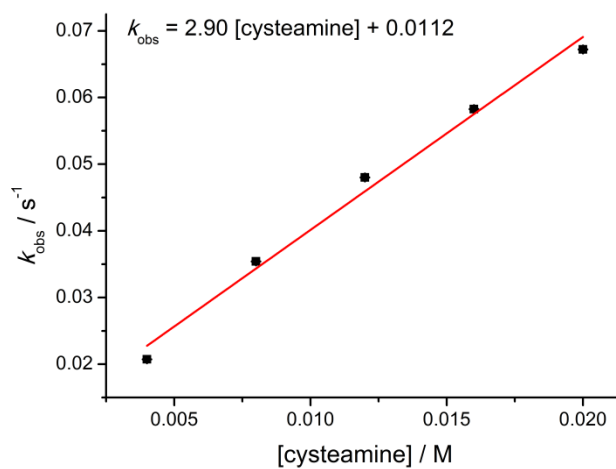
Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean
Abs3	0.00240	1.37×10^{-3}	1.38×10^{-3}
Abs4	(60)	1.38×10^{-3}	
Abs5	0.00288	1.52×10^{-3}	1.52×10^{-3}
Abs6	(72)	1.51×10^{-3}	
Abs7	0.00336	1.67×10^{-3}	1.67×10^{-3}
Abs8	(84)	1.66×10^{-3}	
Abs9	0.00384	1.78×10^{-3}	1.80×10^{-3}
Abs10	(96)	1.82×10^{-3}	
Abs11	0.00432	1.93×10^{-3}	1.98×10^{-3}
Abs12	(108)	2.02×10^{-3}	

$k_2 = 0.308 \text{ M}^{-1} \text{ s}^{-1}$

 α -Br-TMC (73)

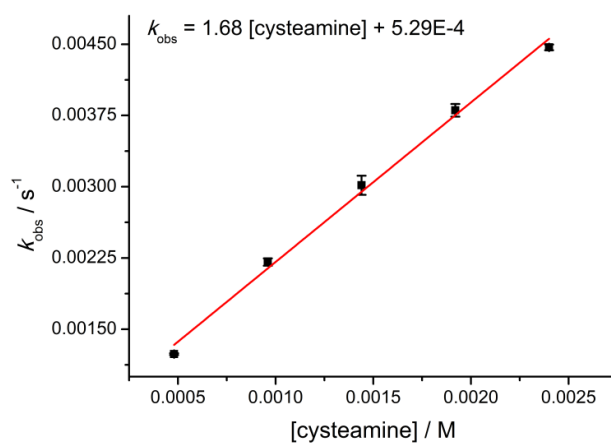
Entry	Thiol [M] (fold)	k_{obs} [s ⁻¹] Mean of replicates
1	0.00400 (100)	0.0207
2	0.00800 (200)	0.0354
3	0.0120 (300)	0.0480
4	0.0160 (400)	0.0583
5	0.0200 (500)	0.0672

$k_2 = 2.90 \text{ M}^{-1} \text{ s}^{-1}$

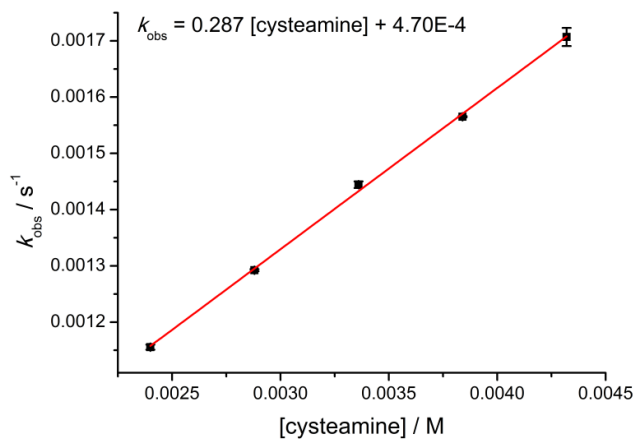


α -Cl-TMC (74)

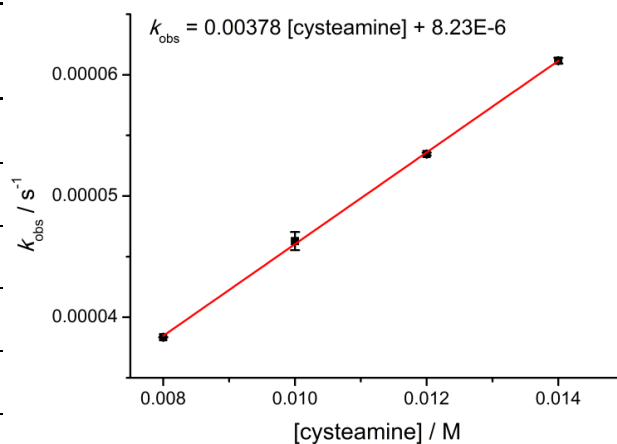
Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean
Abs3	0.00480	1.23×10^{-3}	1.24×10^{-3}
Abs4	(12)	1.25×10^{-3}	
Abs5	0.00960	2.17×10^{-3}	2.21×10^{-3}
Abs6	(24)	2.25×10^{-3}	
Abs7	0.0144	3.12×10^{-3}	3.02×10^{-3}
Abs8	(36)	2.91×10^{-3}	
Abs9	0.0192	3.87×10^{-3}	3.80×10^{-3}
Abs10	(48)	3.74×10^{-3}	
Abs11	0.0240	4.44×10^{-3}	4.47×10^{-3}
Abs12	(60)	4.50×10^{-3}	
$k_2 = 1.68 \text{ M}^{-1} \text{ s}^{-1}$			

 α -I-TMC (75)

Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean
Abs3	0.00240	1.15×10^{-3}	1.16×10^{-3}
Abs4	(60)	1.16×10^{-3}	
Abs5	0.00288	1.29×10^{-3}	1.29×10^{-3}
Abs6	(72)	1.29×10^{-3}	
Abs7	0.00336	1.45×10^{-3}	1.44×10^{-3}
Abs8	(84)	1.44×10^{-3}	
Abs9	0.00384	1.56×10^{-3}	1.57×10^{-3}
Abs10	(96)	1.57×10^{-3}	
Abs11	0.00432	1.69×10^{-3}	1.71×10^{-3}
Abs12	(108)	1.72×10^{-3}	
$k_2 = 0.287 \text{ M}^{-1} \text{ s}^{-1}$			

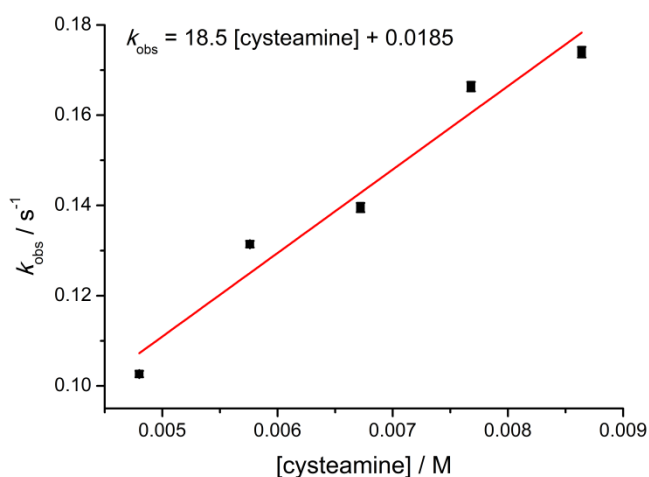
 α -COOH-TMC (76)

Entry	Thiol [M] (fold)	Individual k_{obs} [s^{-1}]	k_{obs} [s^{-1}] Mean
Abs3	0.00800	3.83×10^{-5}	3.84×10^{-5}
Abs4	(200)	3.84×10^{-5}	
Abs5	0.0100	4.70×10^{-5}	4.63×10^{-5}
Abs6	(250)	4.55×10^{-5}	
Abs7	0.0120	5.36×10^{-5}	5.35×10^{-5}
Abs8	(300)	5.33×10^{-5}	
Abs9	0.0140	6.09×10^{-5}	6.12×10^{-5}
Abs10	(350)	6.14×10^{-5}	
Abs11	0.160	---	---
Abs12	(400)	---	---
$k_2 = 0.00378 \text{ M}^{-1} \text{ s}^{-1}$			



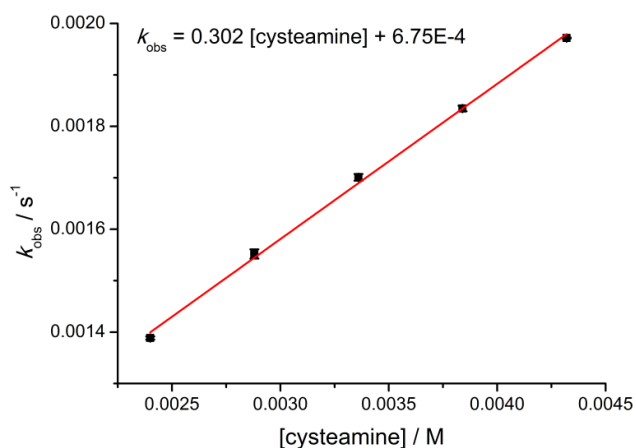
α -CF₃-TMC (77)

Entry	Thiol [M] (fold)	k_{obs} [s ⁻¹] Mean of replicates
1	0.00480 (60)	0.103
2	0.00576 (72)	0.131
3	0.00672 (84)	0.140
4	0.00768 (96)	0.166
5	0.00864 (108)	0.174
$k_2 = 18.5 \text{ M}^{-1} \text{ s}^{-1}$		



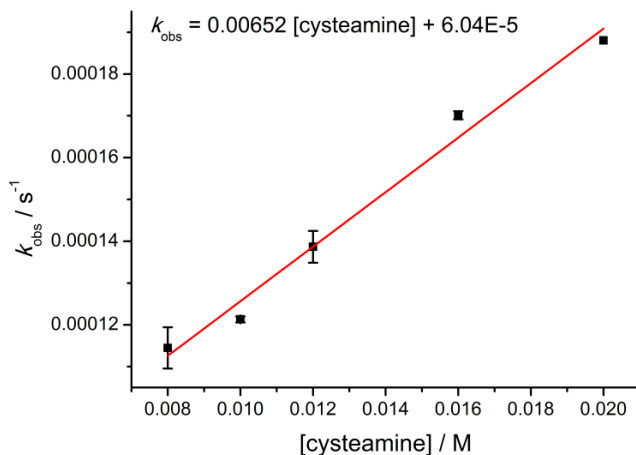
α -p-NO₂-C₆H₄-TMC (78)

Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean
Abs3	0.00240	1.39×10^{-3}	1.39×10^{-3}
Abs4	(60)	1.39×10^{-3}	
Abs5	0.00288	1.56×10^{-3}	1.55×10^{-3}
Abs6	(72)	1.54×10^{-3}	
Abs7	0.00336	1.70×10^{-3}	1.70×10^{-3}
Abs8	(84)	1.69×10^{-3}	
Abs9	0.00384	1.84×10^{-3}	1.83×10^{-3}
Abs10	(96)	1.83×10^{-3}	
Abs11	0.00432	1.97×10^{-3}	1.97×10^{-3}
Abs12	(108)	1.97×10^{-3}	
$k_2 = 0.302 \text{ M}^{-1} \text{ s}^{-1}$			



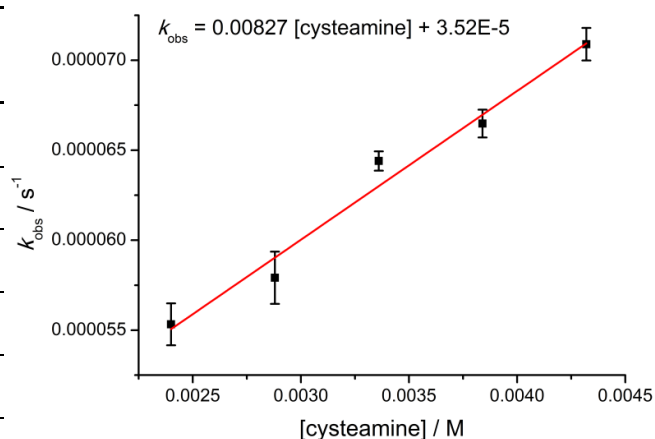
α -Ph-TMC (79)

Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean
Abs3	0.00800	1.19×10^{-4}	1.14×10^{-4}
Abs4	(200)	1.10×10^{-4}	
Abs5	0.0100	1.22×10^{-4}	1.21×10^{-4}
Abs6	(250)	1.21×10^{-4}	
Abs7	0.0120	1.35×10^{-4}	1.39×10^{-4}
Abs8	(300)	1.42×10^{-4}	
Abs9	0.0160	1.69×10^{-4}	1.70×10^{-4}
Abs10	(400)	1.71×10^{-4}	
Abs11	0.0200	1.88×10^{-4}	1.88×10^{-4}
Abs12	(500)	---	
$k_2 = 0.00652 \text{ M}^{-1} \text{ s}^{-1}$			

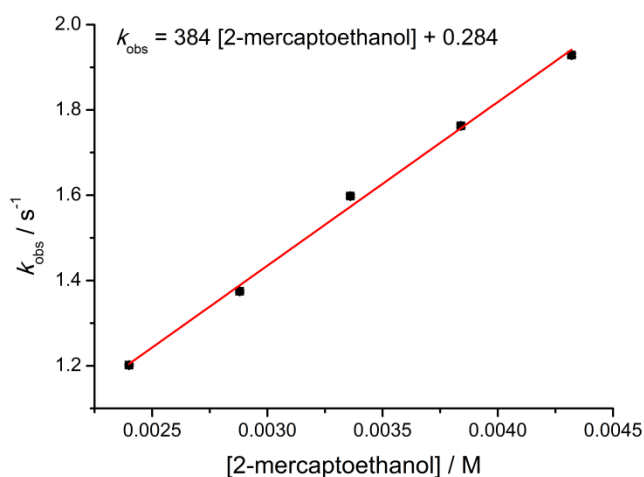


α -*p*-OMe-C₆H₄-TMC (**80**)

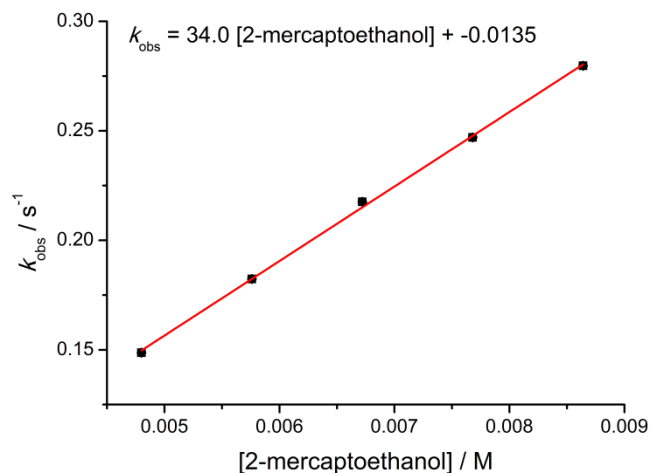
Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean s
Abs3	0.00240	5.65×10^{-5}	5.53×10^{-5}
Abs4	(60)	5.42×10^{-5}	
Abs5	0.00288	5.94×10^{-5}	5.79×10^{-5}
Abs6	(72)	5.65×10^{-5}	
Abs7	0.00336	6.49×10^{-5}	6.44×10^{-5}
Abs8	(84)	6.39×10^{-5}	
Abs9	0.00384	6.73×10^{-5}	6.65×10^{-5}
Abs10	(96)	6.57×10^{-5}	
Abs11	0.00432	7.18×10^{-5}	7.09×10^{-5}
Abs12	(108)	7.00×10^{-5}	
$k_2 = 0.00827 \text{ M}^{-1} \text{ s}^{-1}$			

7.4.3. Representative k_2 value determinations for selected α -X-TMCs with other thiols α -CN-TMC (**69**) with 2-mercaptoethanol

Entry	Thiol [M] (fold)	k_{obs} [s ⁻¹] Mean of replicates
1	0.00240 (60)	1.20
2	0.00288 (72)	1.37
3	0.00336 (84)	1.60
4	0.00384 (96)	1.76
5	0.00432 (108)	1.93
$k_2 = 384 \text{ M}^{-1} \text{ s}^{-1}$		

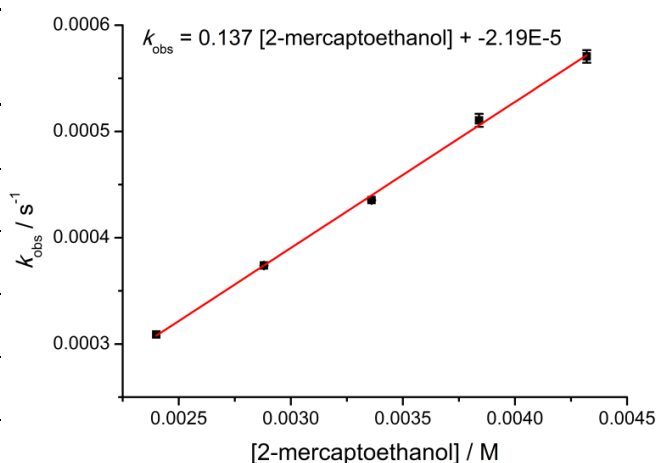
 α -NO₂-TMC (**71**) with 2-mercaptoethanol

Entry	Thiol [M] (fold)	k_{obs} [s ⁻¹] Mean of replicates
1	0.00480 (60)	0.149
2	0.00576 (72)	0.182
3	0.00672 (84)	0.218
4	0.00768 (96)	0.247
5	0.00864 (108)	0.280
$k_2 = 34.0 \text{ M}^{-1} \text{ s}^{-1}$		

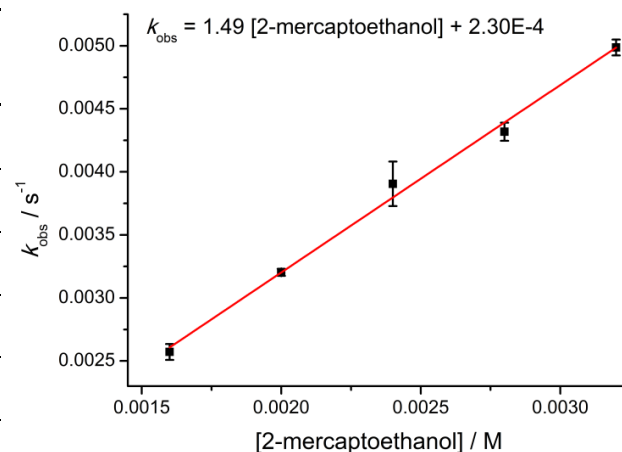


α -Br-TMC (**73**) with 2-mercaptoethanol

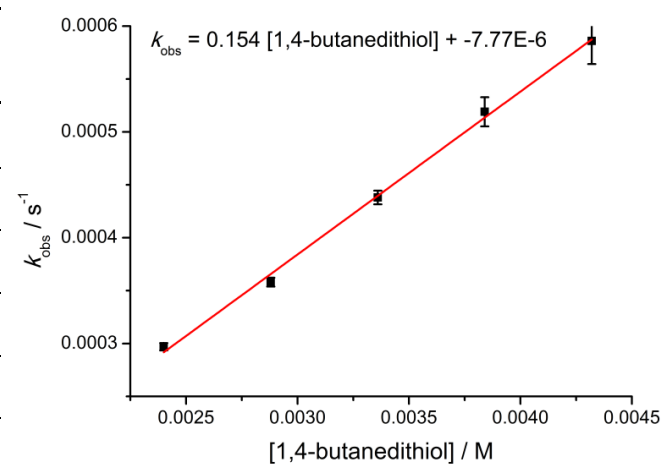
Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean
Abs3	0.00240	---	3.09 x 10 ⁻⁴
Abs4	(60)	3.09 x 10 ⁻⁴	
Abs5	0.00288	3.74 x 10 ⁻⁴	3.74 x 10 ⁻⁴
Abs6	(72)	3.74 x 10 ⁻⁴	
Abs7	0.00336	4.35 x 10 ⁻⁴	4.35 x 10 ⁻⁴
Abs8	(84)	4.36 x 10 ⁻⁴	
Abs9	0.00384	5.04 x 10 ⁻⁴	5.10 x 10 ⁻⁴
Abs10	(96)	5.17 x 10 ⁻⁴	
Abs11	0.00432	5.77 x 10 ⁻⁴	5.71 x 10 ⁻⁴
Abs12	(108)	5.65 x 10 ⁻⁴	
$k_2 = 0.137 \text{ M}^{-1} \text{ s}^{-1}$			

 α -CF₃-TMC (**77**) with 2-mercaptoethanol

Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean
Abs3	0.00160	2.51×10^{-3}	2.57×10^{-3}
Abs4	(20)	2.63×10^{-3}	
Abs5	0.00200	3.17×10^{-3}	3.20×10^{-3}
Abs6	(25)	3.23×10^{-3}	
Abs7	0.00240	4.08×10^{-3}	3.90×10^{-3}
Abs8	(30)	3.73×10^{-3}	
Abs9	0.00280	4.39×10^{-3}	4.32×10^{-3}
Abs10	(35)	4.25×10^{-3}	
Abs11	0.00320	4.92×10^{-3}	4.99×10^{-3}
Abs12	(40)	5.05×10^{-3}	
$k_2 = 1.49 \text{ M}^{-1} \text{ s}^{-1}$			

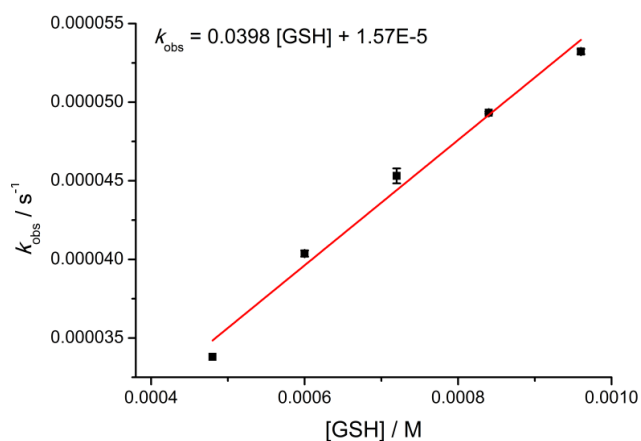
 α -Br-TMC (**73**) with 1,4-butanedithiol

Entry	Thiol [M] (fold)	Individual k_{obs} [s ⁻¹]	k_{obs} [s ⁻¹] Mean
Abs3	0.00240	2.94×10^{-4}	2.97×10^{-4}
Abs4	(60)	3.01×10^{-4}	
Abs5	0.00288	3.63×10^{-4}	3.58×10^{-4}
Abs6	(72)	3.54×10^{-4}	
Abs7	0.00336	4.31×10^{-4}	4.38×10^{-4}
Abs8	(84)	4.44×10^{-4}	
Abs9	0.00384	5.05×10^{-4}	5.19×10^{-4}
Abs10	(96)	5.32×10^{-4}	
Abs11	0.00432	5.64×10^{-4}	5.86×10^{-4}
Abs12	(108)	6.08×10^{-4}	
$k_2 = 0.154 \text{ M}^{-1} \text{ s}^{-1}$			

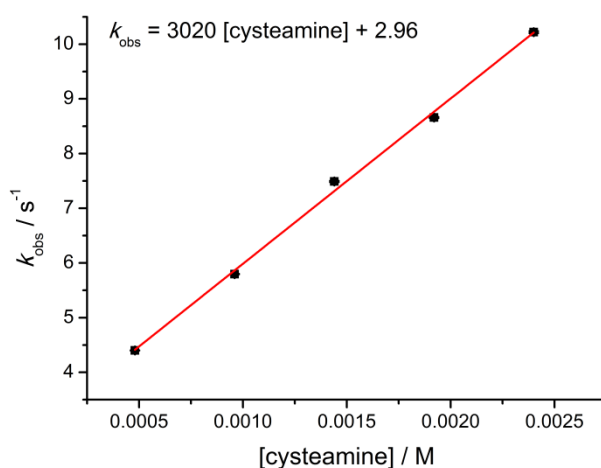


α -Br-TMC (73) with glutathione

Entry	Thiol [M] (fold)	Individual k_{obs} [s^{-1}]	k_{obs} [s^{-1}] Mean of duplicates
Abs3	0.000480	---	3.38×10^{-5}
Abs4	(12)	3.38×10^{-5}	
Abs5	0.000600	4.06×10^{-5}	4.04×10^{-5}
Abs6	(15)	4.02×10^{-5}	
Abs7	0.000720	4.58×10^{-5}	4.53×10^{-5}
Abs8	(18)	4.48×10^{-5}	
Abs9	0.000840	4.93×10^{-5}	4.93×10^{-5}
Abs10	(21)	4.94×10^{-5}	
Abs11	0.000960	5.31×10^{-5}	5.32×10^{-5}
Abs12	(24)	5.34×10^{-5}	
$k_2 = 0.0398 \text{ M}^{-1} \text{ s}^{-1}$			

7.4.4. Representative k_2 value determination for α -CN-pHC (95) with cysteamine

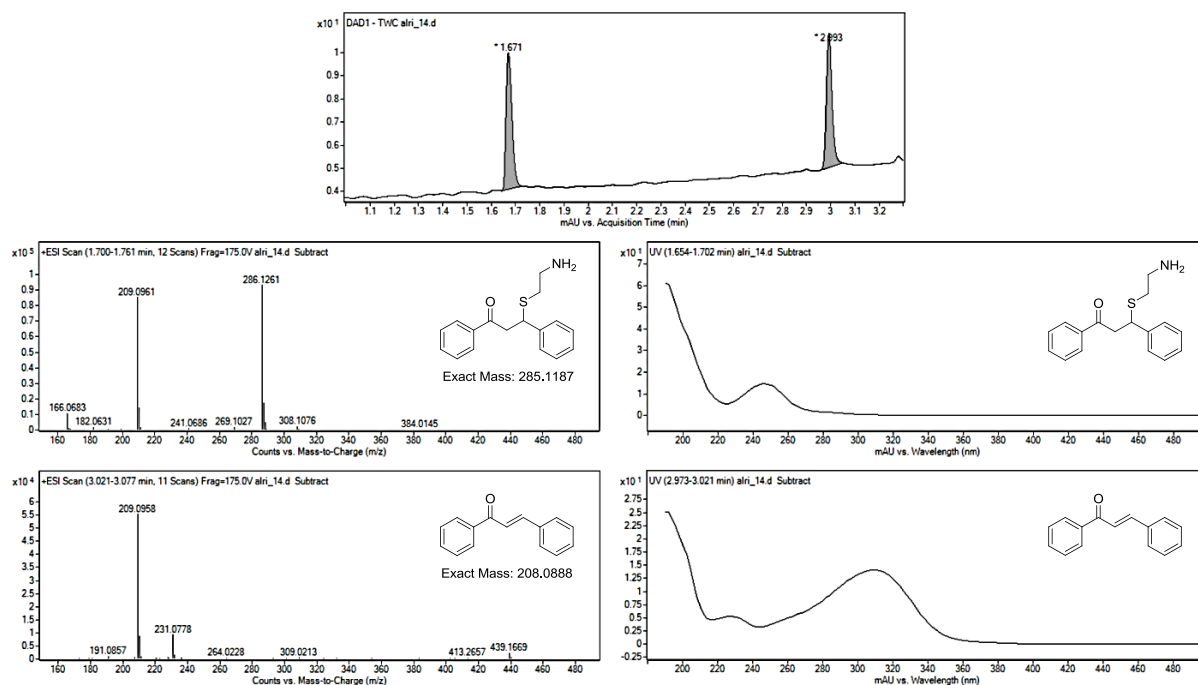
Entry	Thiol [M] (fold)	k_{obs} [s^{-1}] Mean of replicates
1	0.00480 (12)	4.41
2	0.00960 (24)	5.80
3	0.0144 (36)	7.49
4	0.0192 (48)	8.66
5	0.0240 (60)	10.2
$k_2 = 3020 \text{ M}^{-1} \text{ s}^{-1}$		



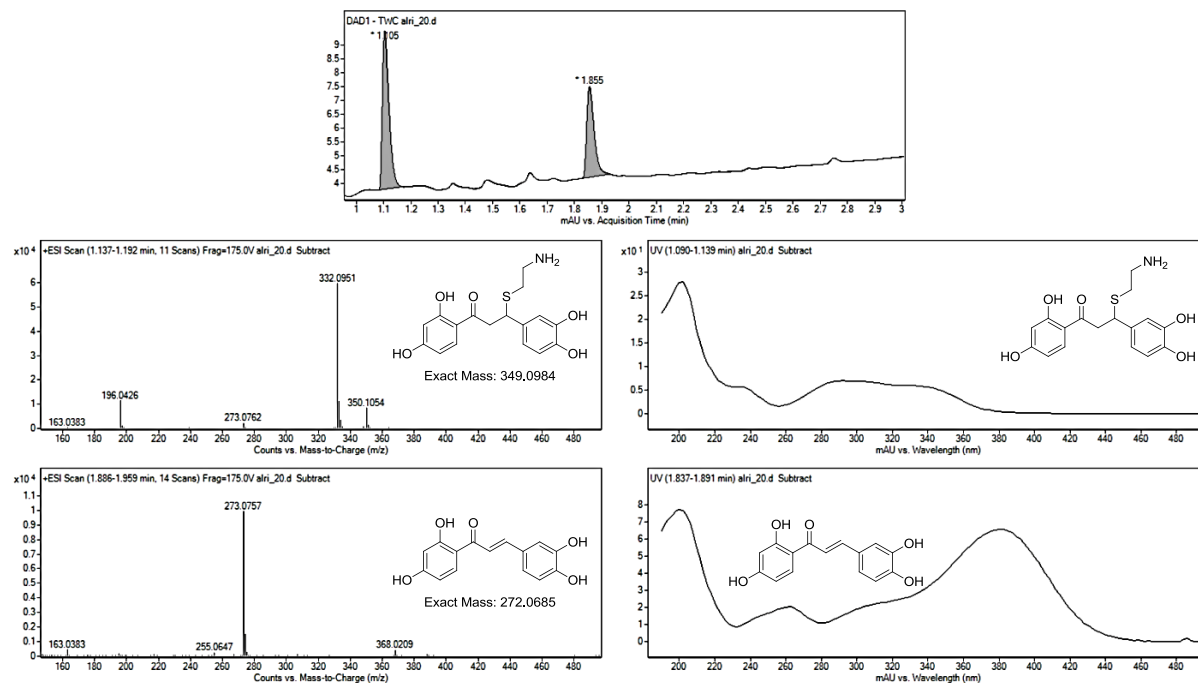
7.5. LC-MS analysis of chalcones in buffer-ethylene glycol solution

7.5.1. α -H-Chalcones in presence of cysteamine

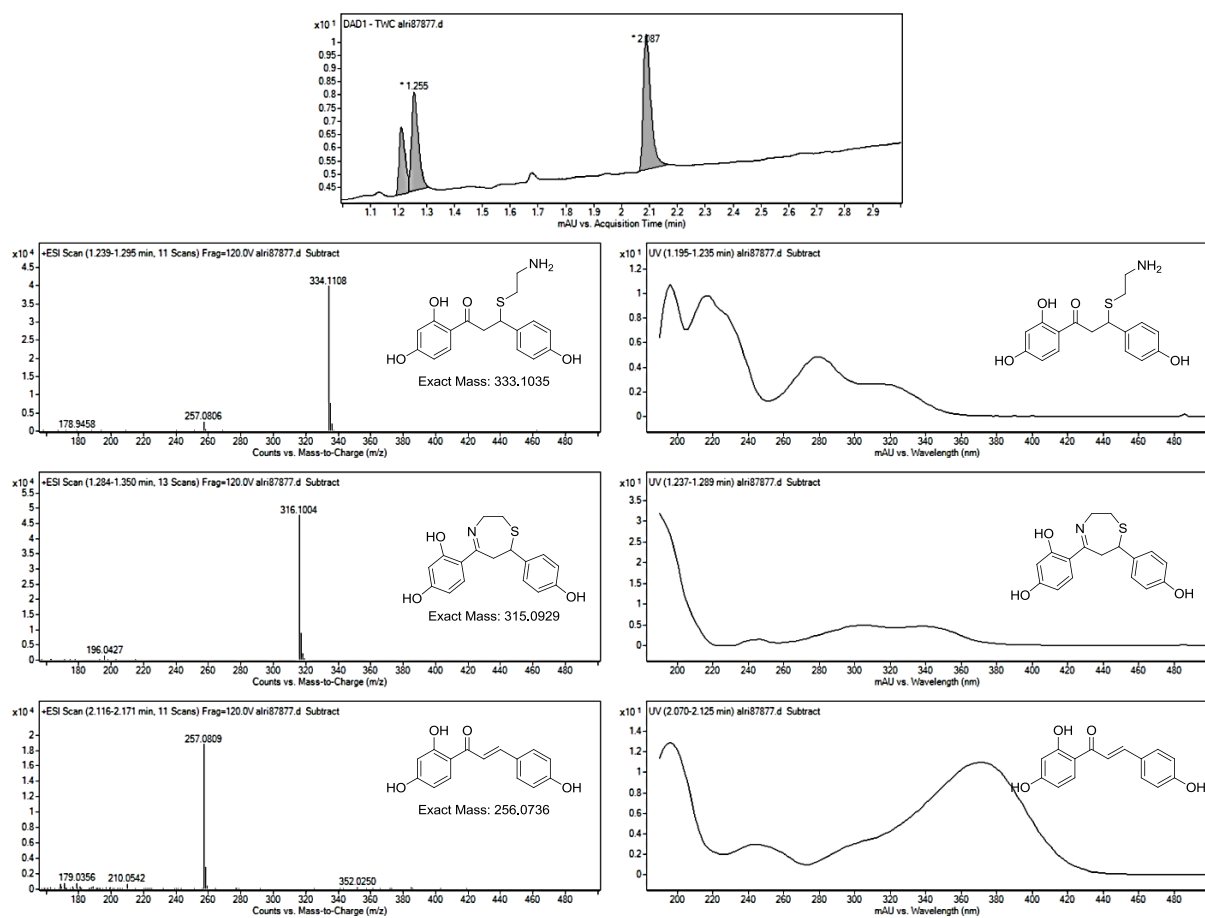
Chalcone (1) with 12 fold cysteamine



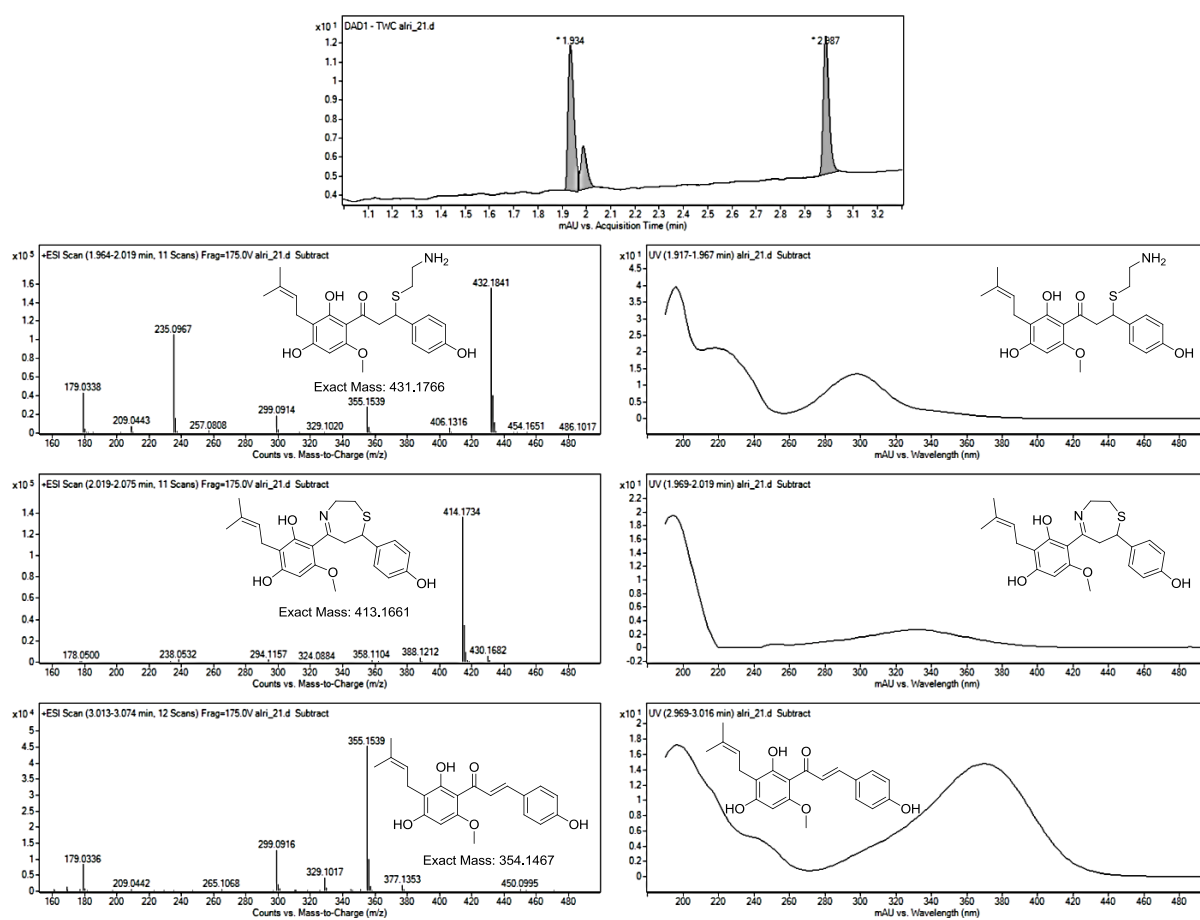
Butein (2) with 60 fold cysteamine



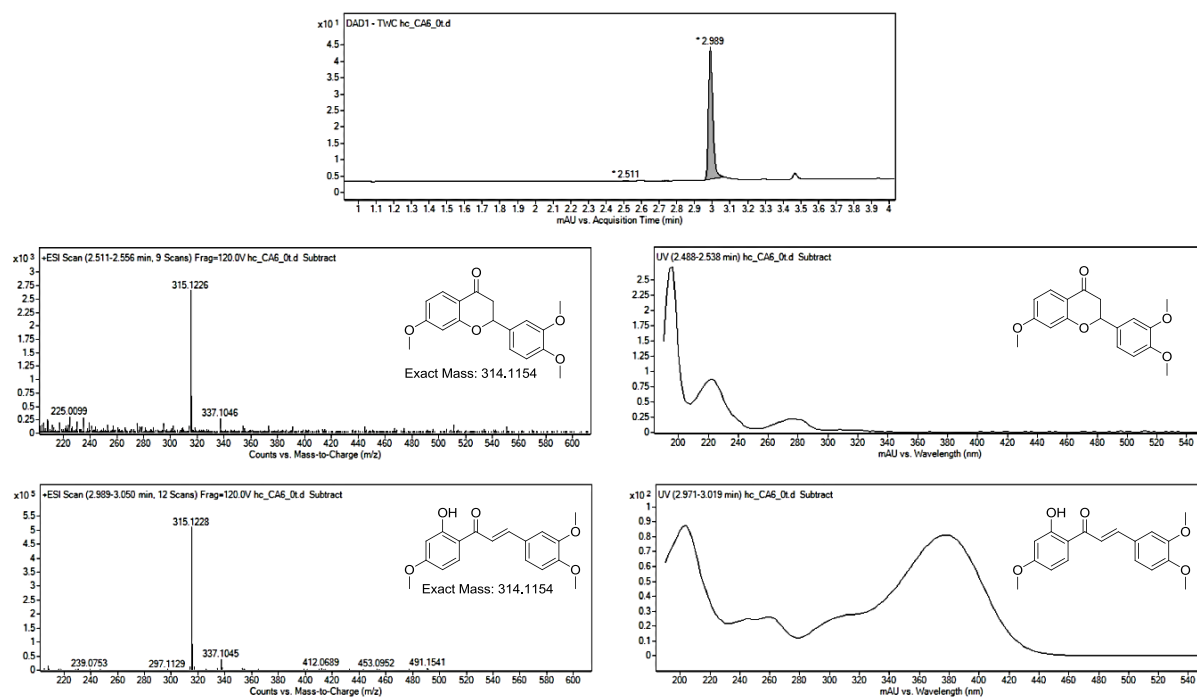
Isoliquiritigenin (4) with 60 fold cysteamine



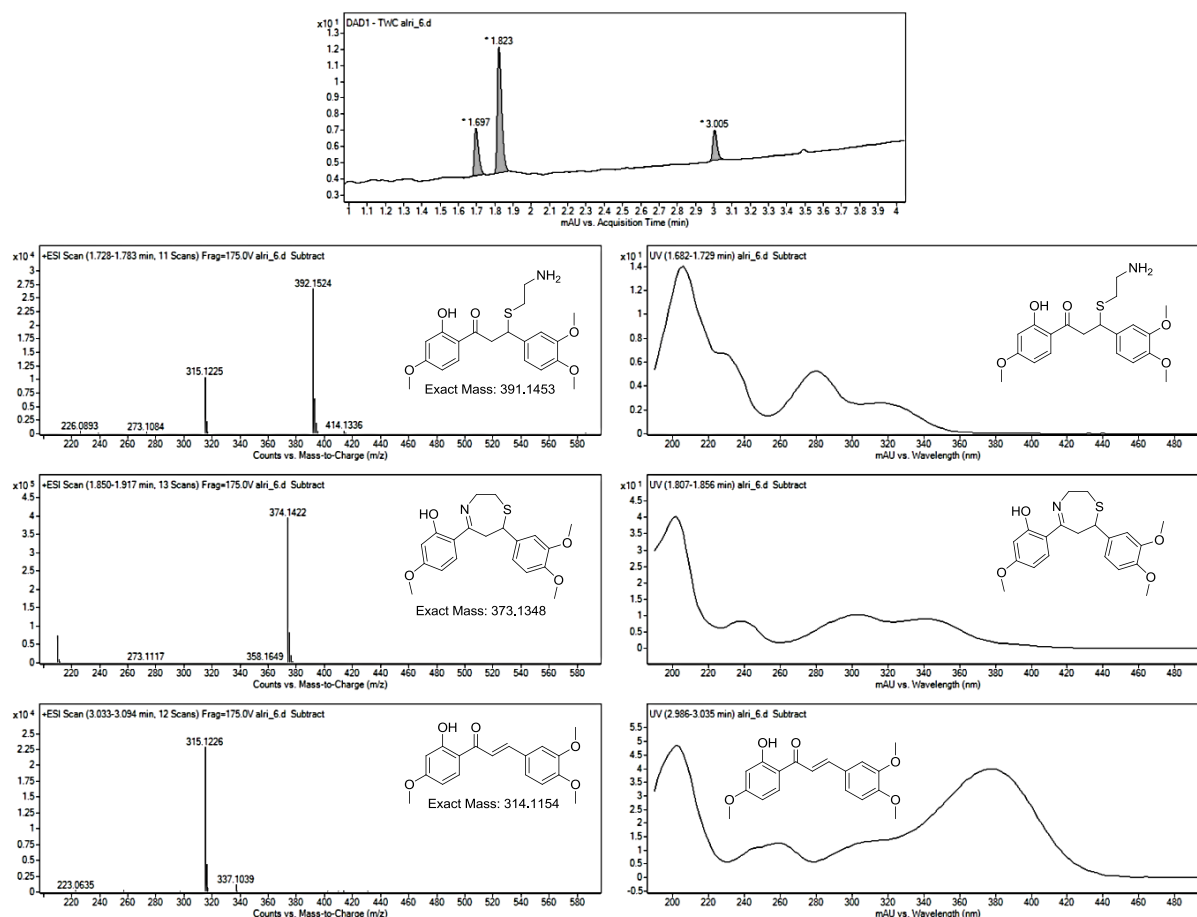
Xanthohumol (5) with 60 fold cysteamine

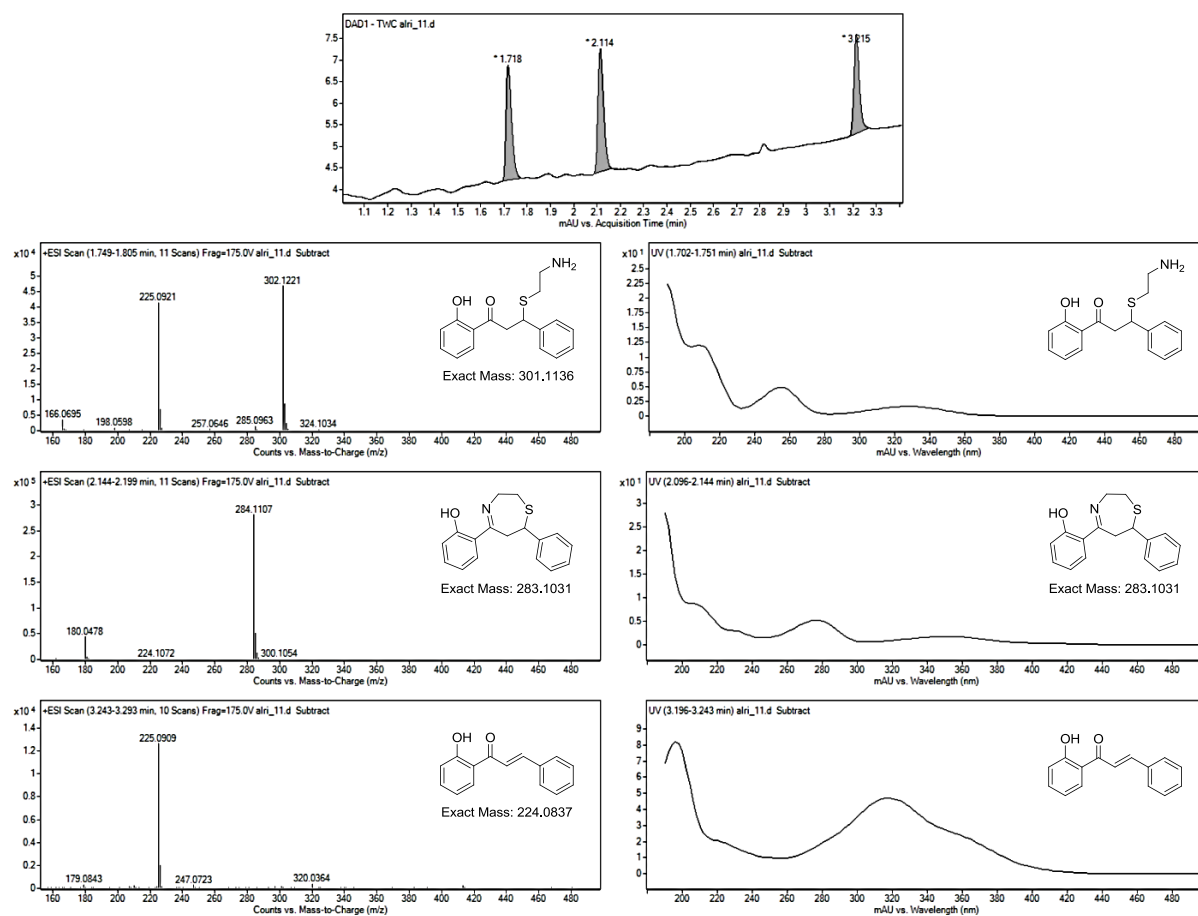


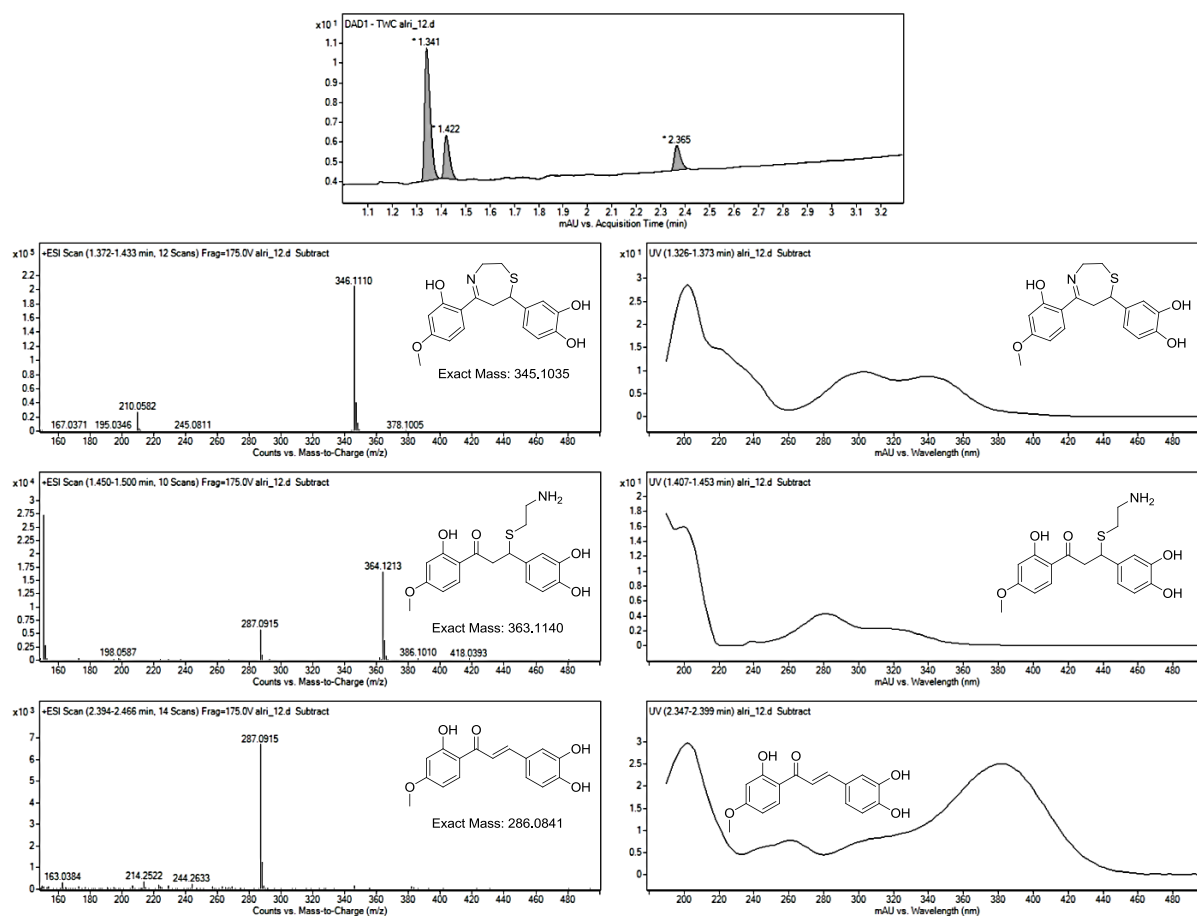
(*E*)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxy-phenyl)prop-2-en-1-one (α -H-HC, **61**) without thiol

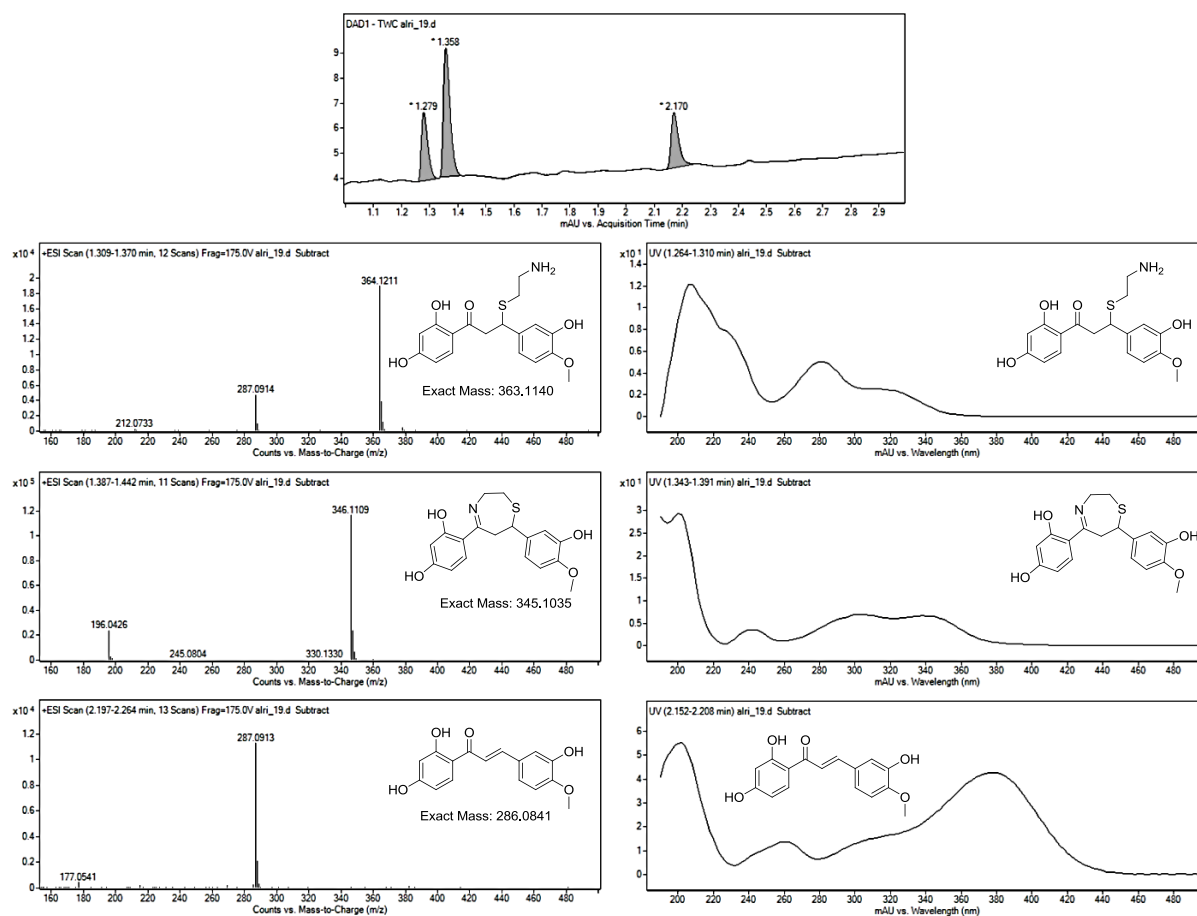


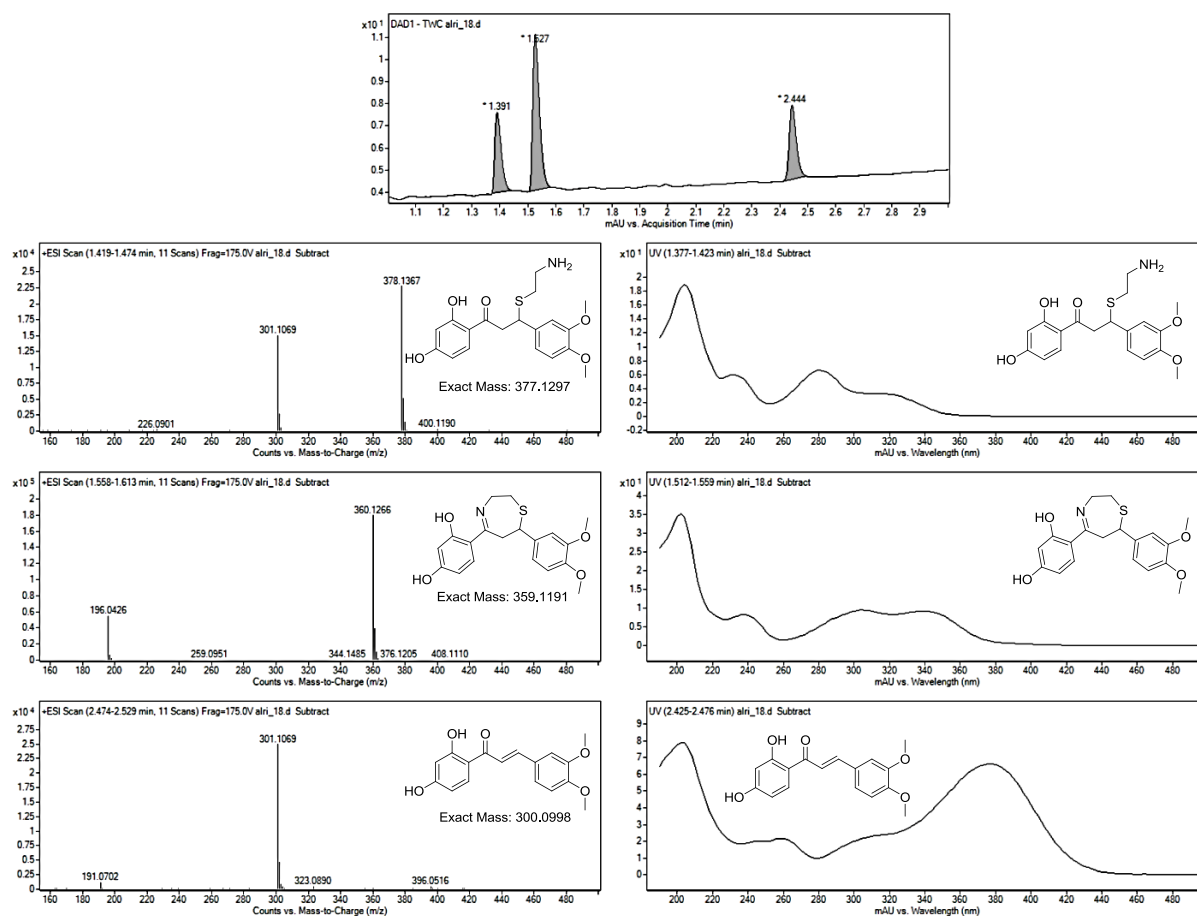
(*E*)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4-methoxy-phenyl)prop-2-en-1-one (α -H-HC (**61**)) with 60 fold cysteamine



(E)-1-(2-Hydroxyphenyl)-3-phenylprop-2-en-1-one (**62**) with 12 fold cysteamine

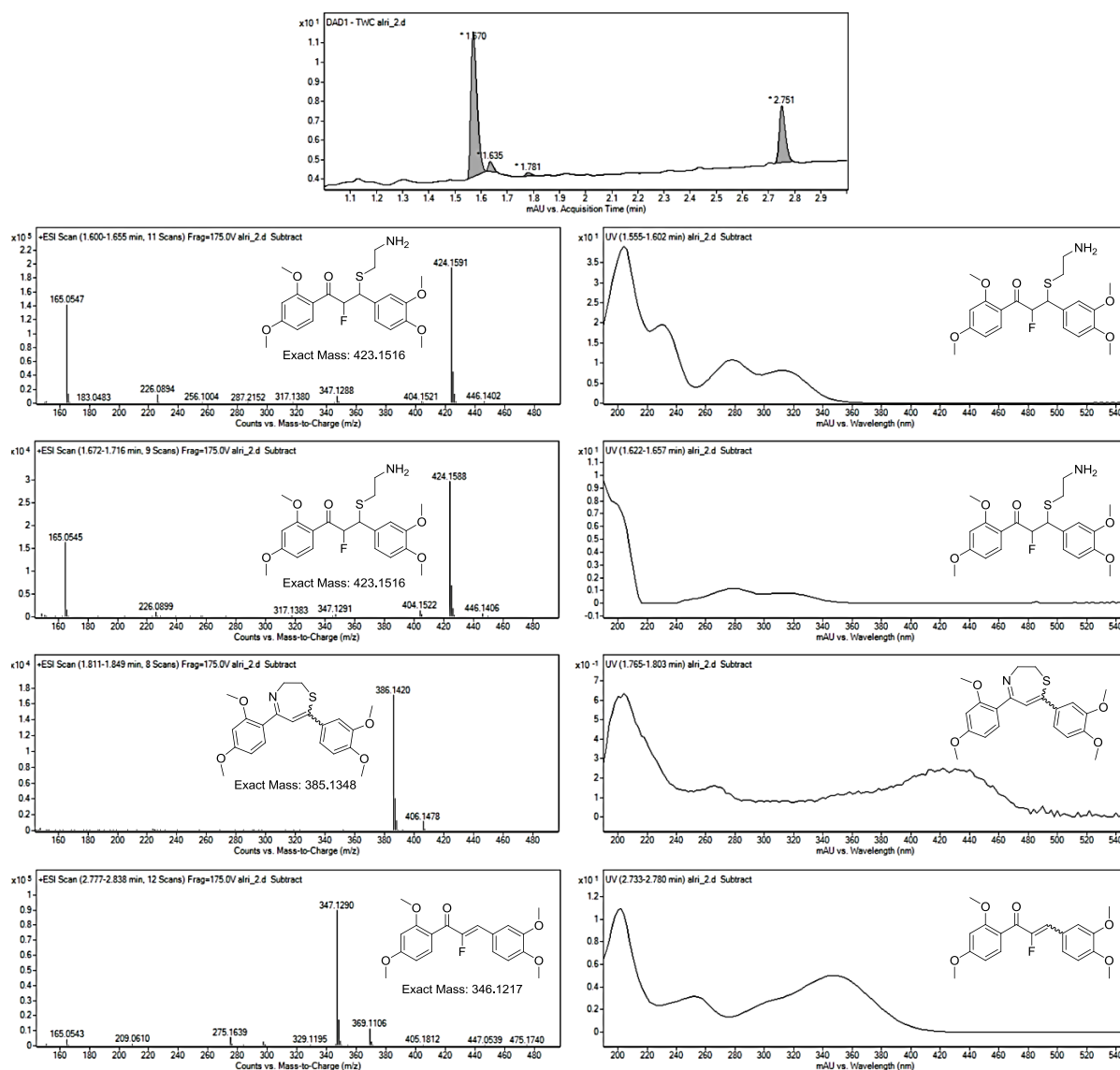
Calythropsin (**116**) with 100 fold cysteamine

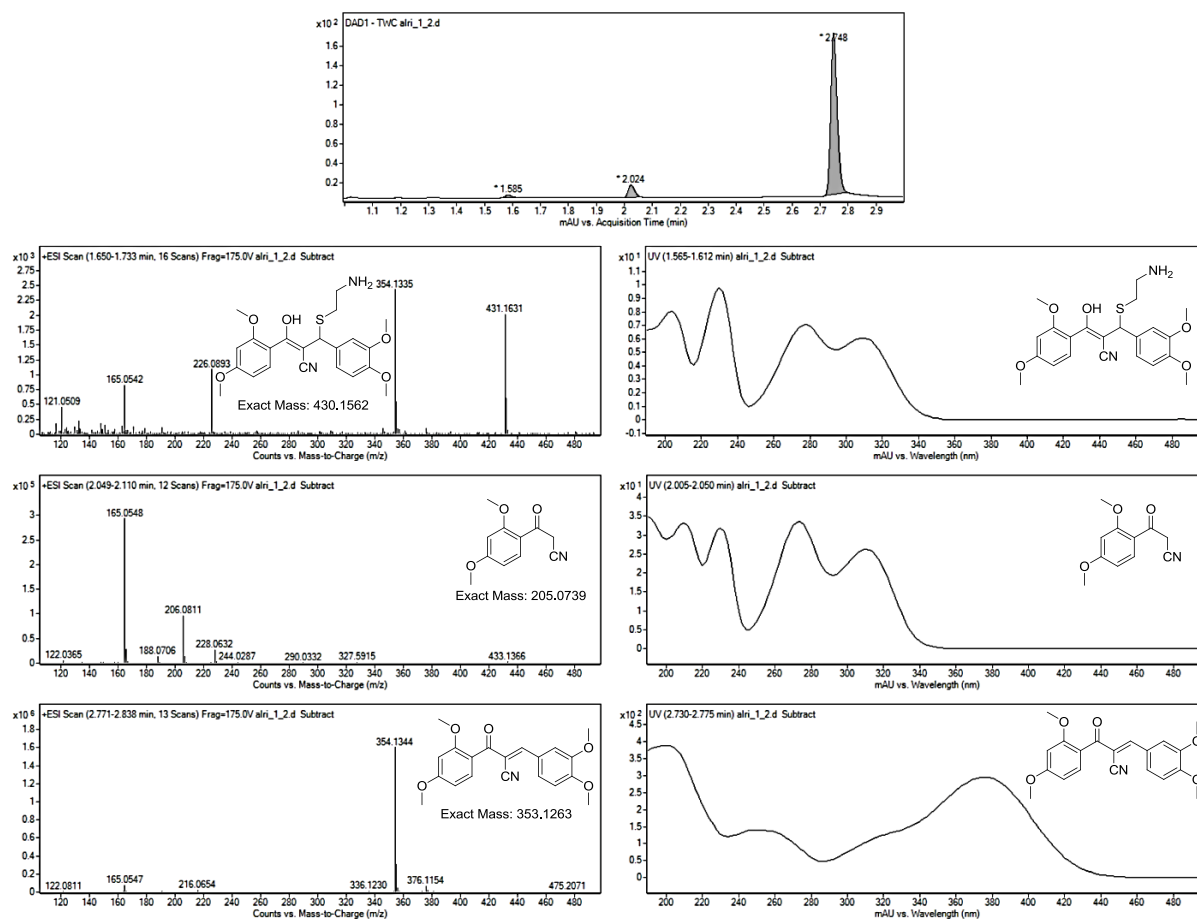
4-Methoxy-2',3,4'-trihydroxychalcone (**118**) with 60 fold cysteamine

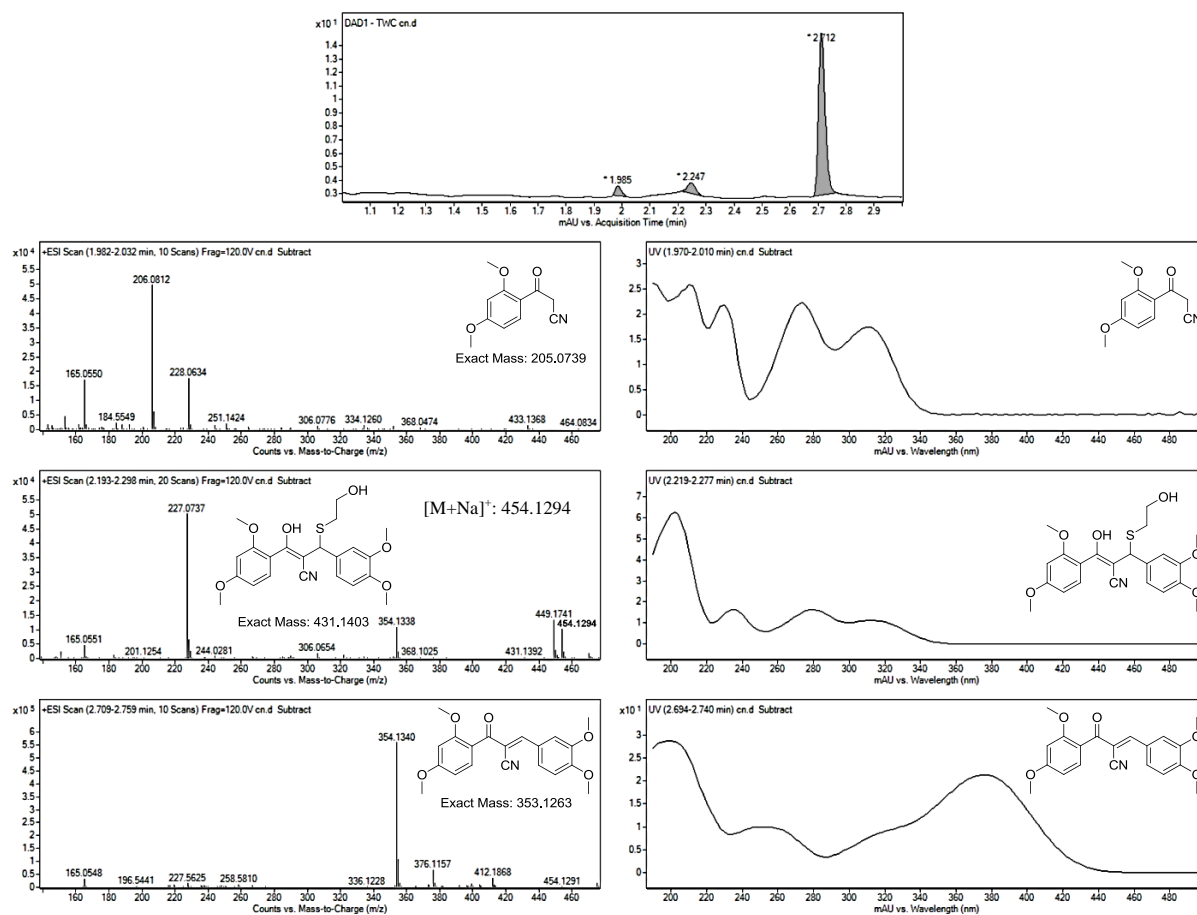
2',4'-Dihydroxy-3,4-dimethoxychalcone (**119**) with 60 fold cysteamine

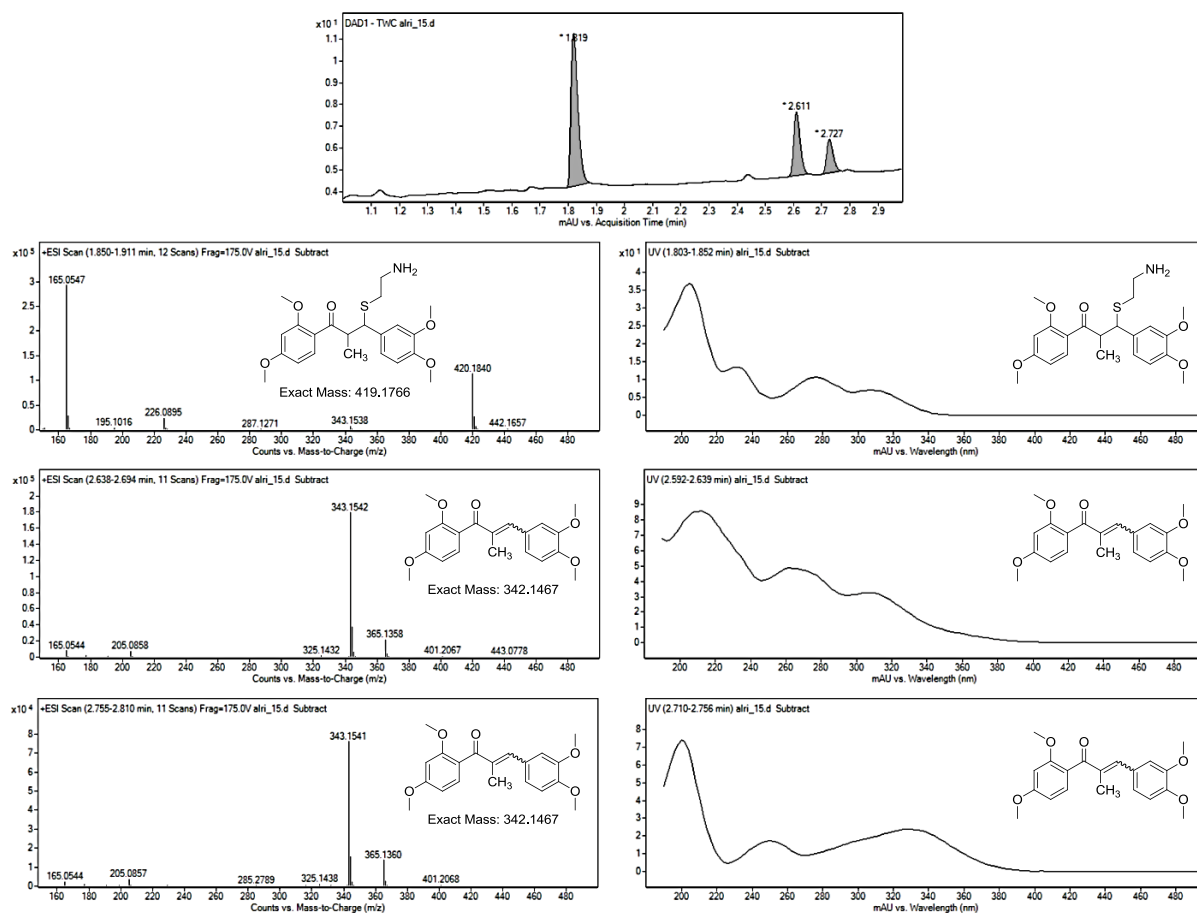
7.5.2. α -X-TMCs in presence of thiols

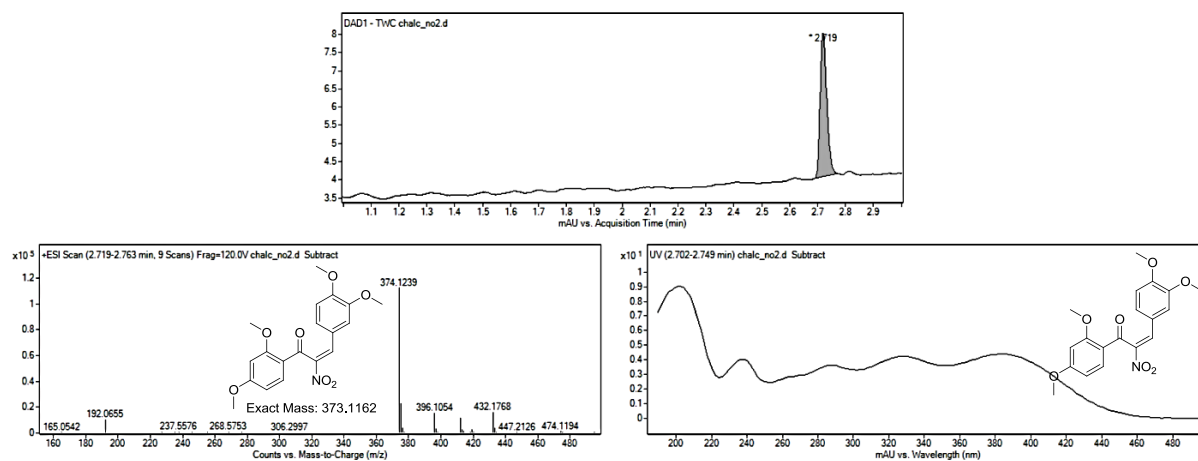
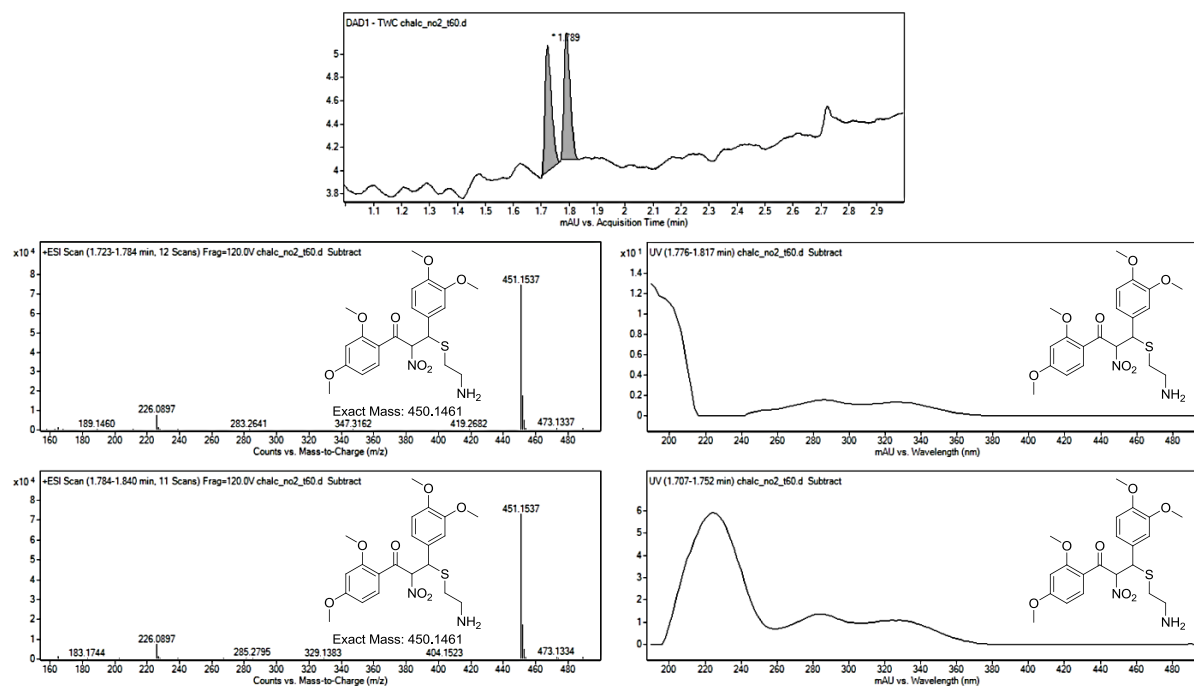
α -F-TMC (**68**) with 200 fold cysteamine

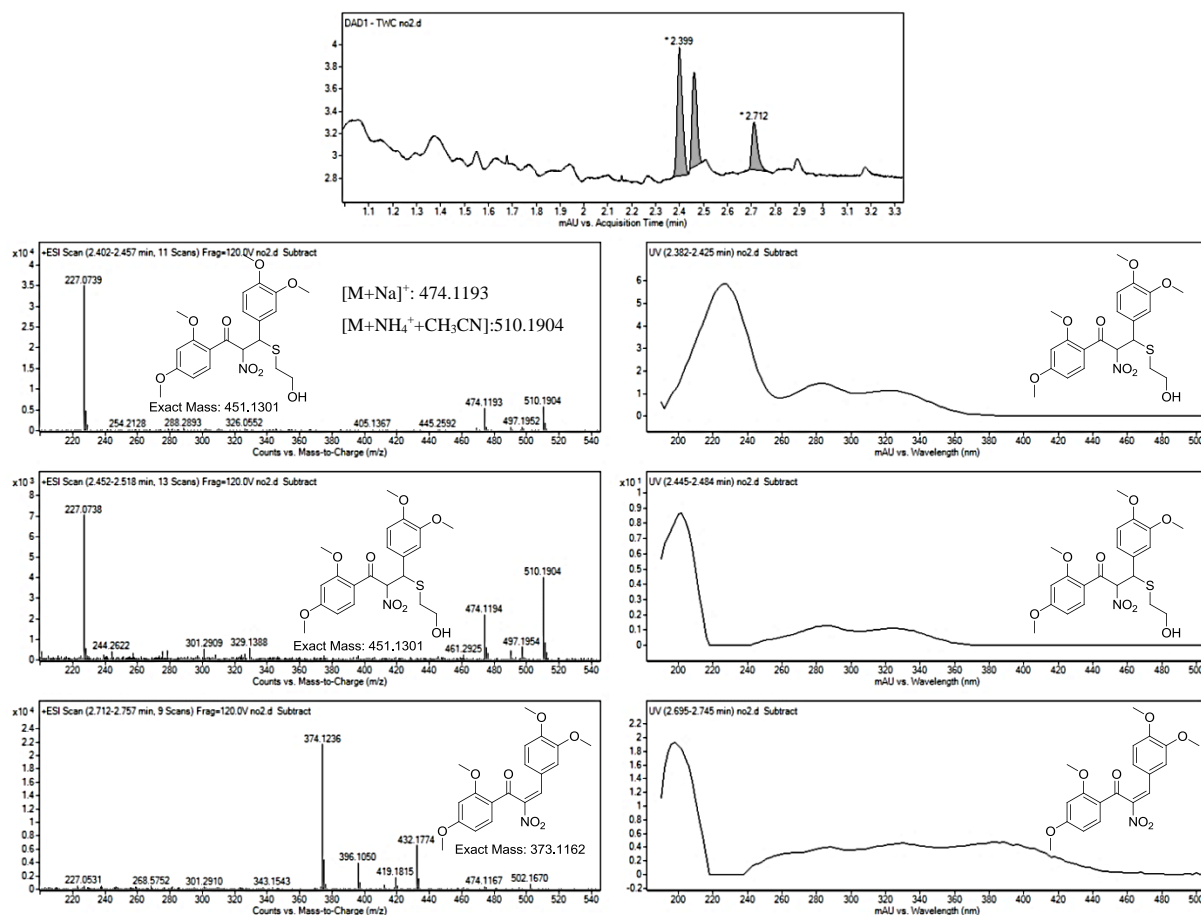


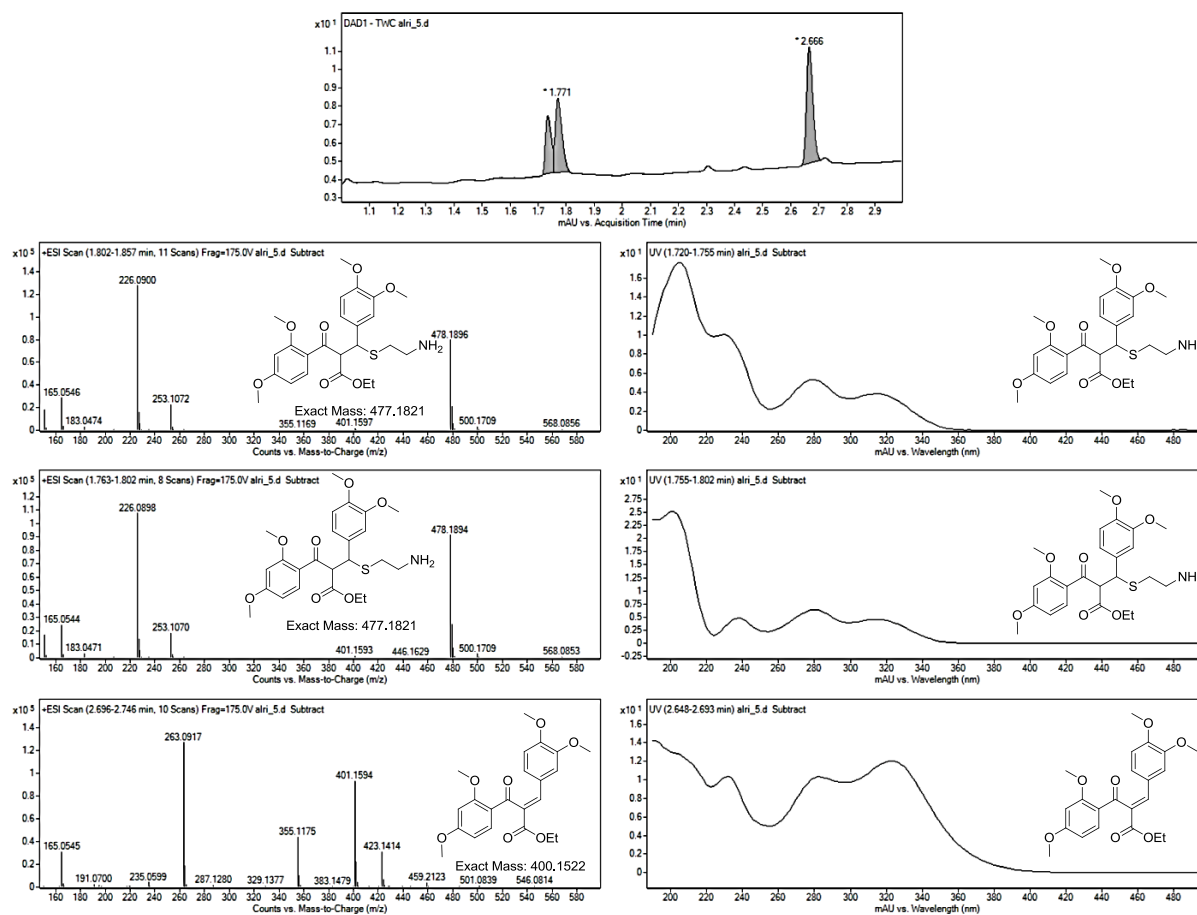
α -CN-TMC (69) with cysteamine

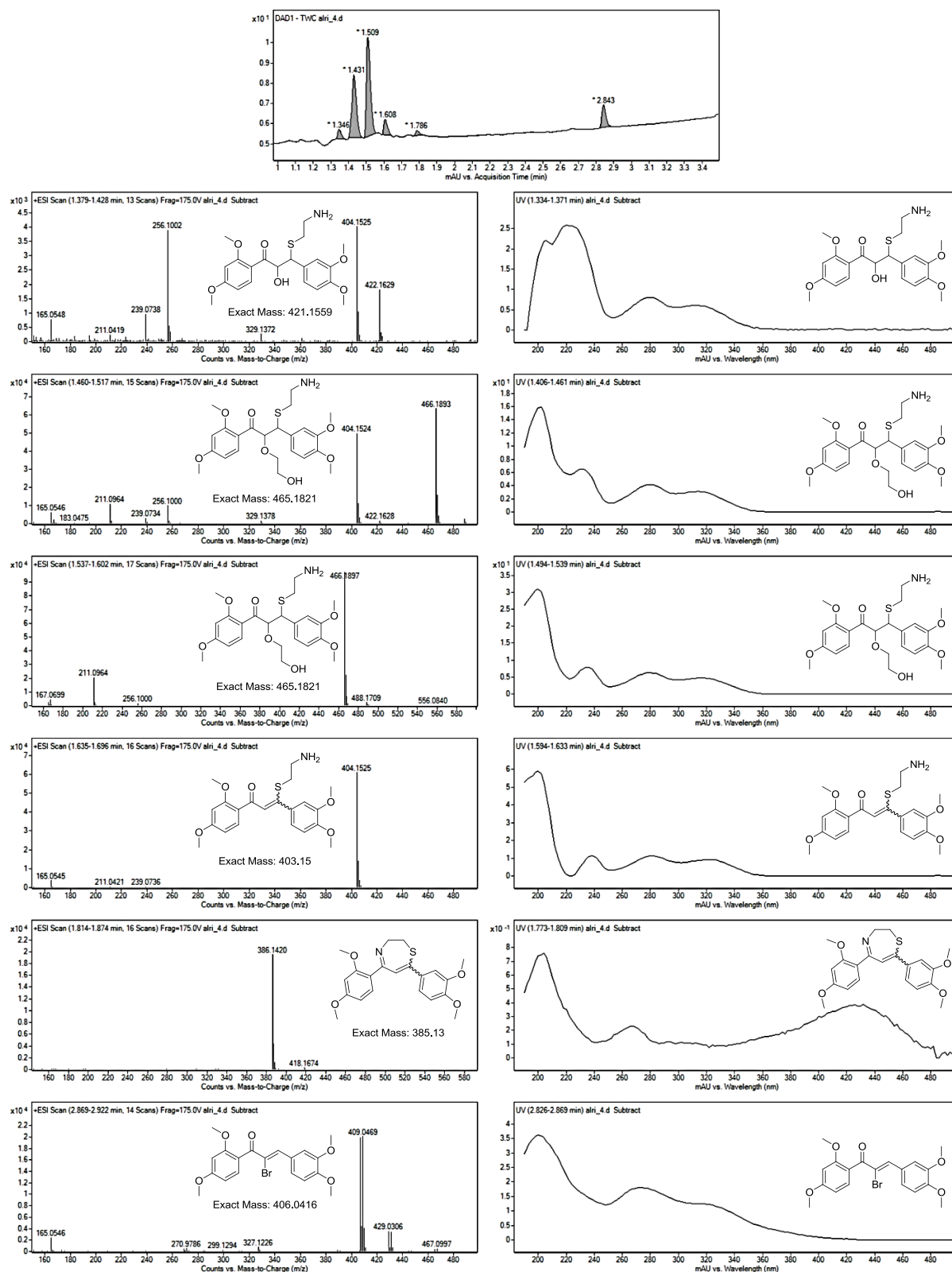
α -CN-TMC (69) with 2-mercaptoethanol

α -Me-TMC (**70**) with 200 fold cysteamine

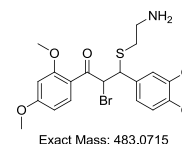
α -NO₂-TMC (**71**) without thiol α -NO₂-TMC (**71**) with 60 fold cysteamine

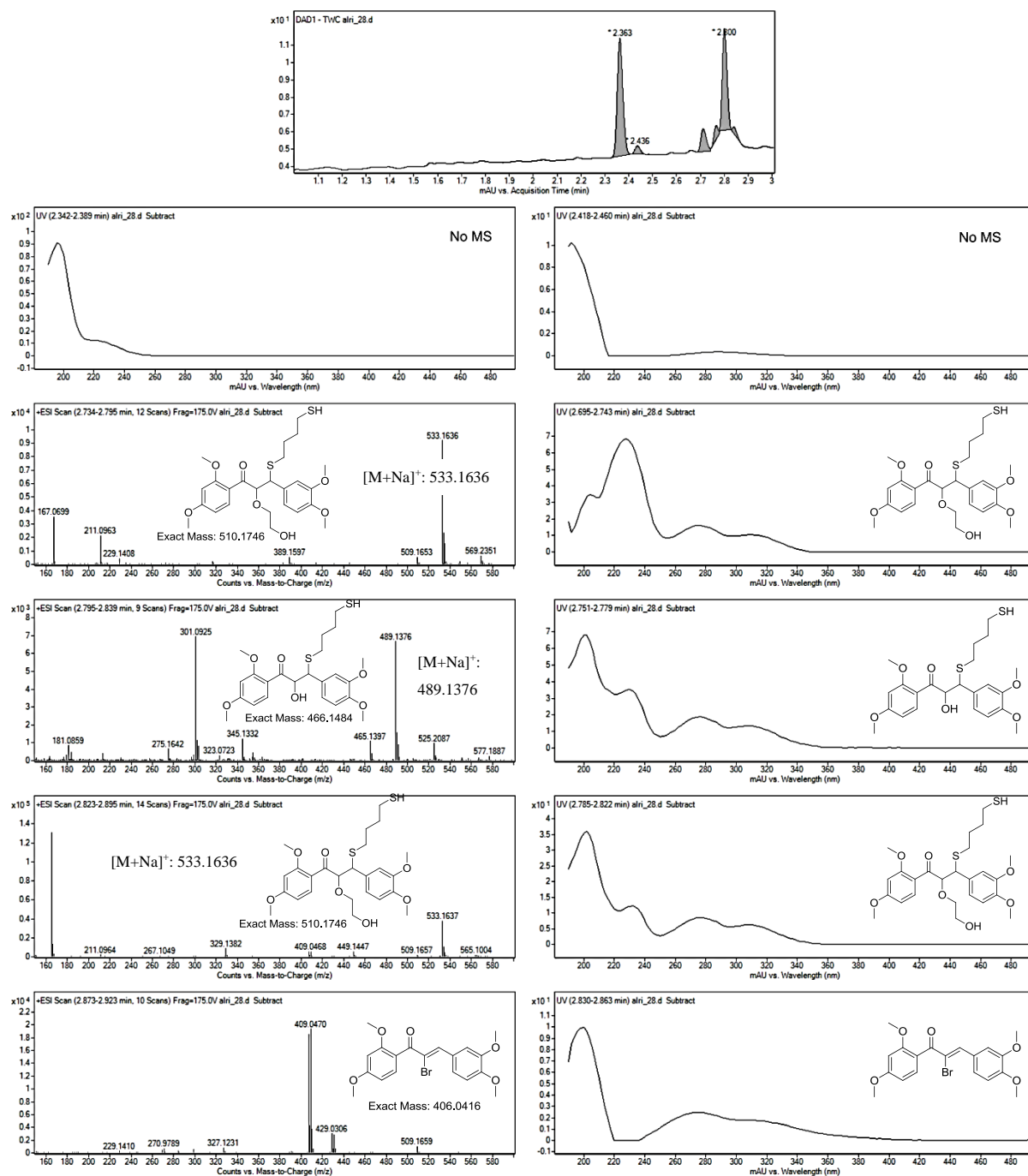
α -NO₂-TMC (**71**) with 2-mercaptoethanol

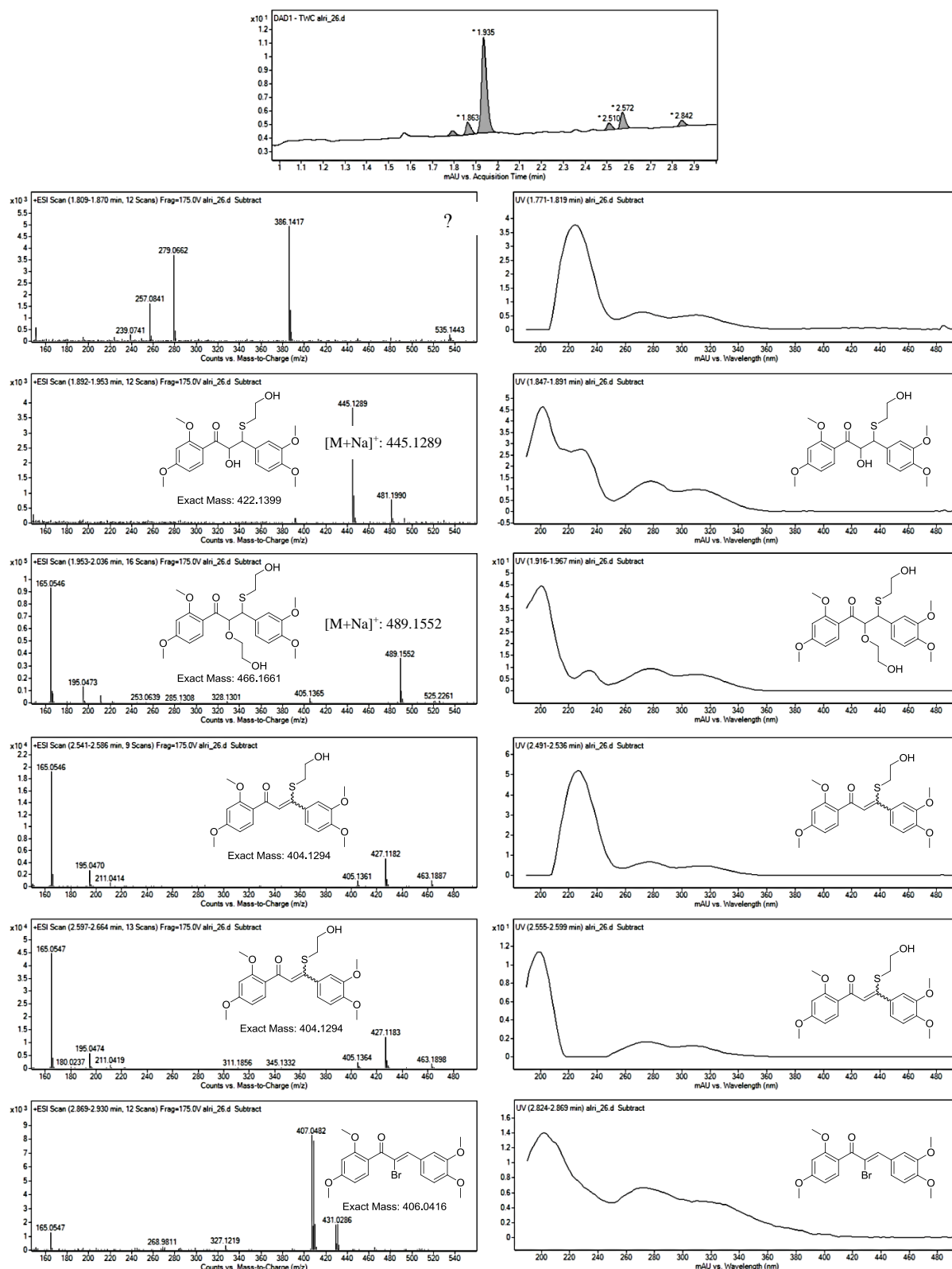
α -COOEt-TMC (**72**) with 60 fold cysteamine

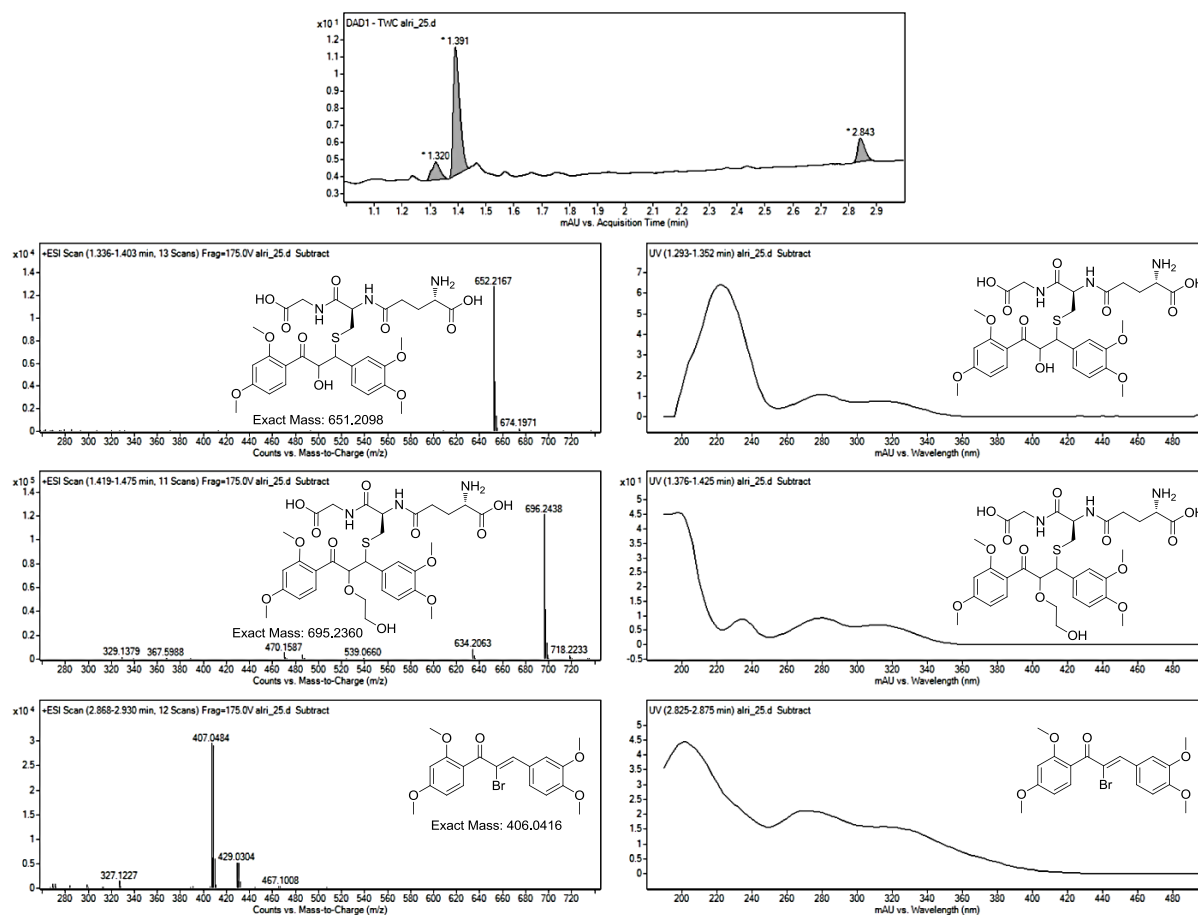
α -Br-TMC (73) with 60 fold cysteamine

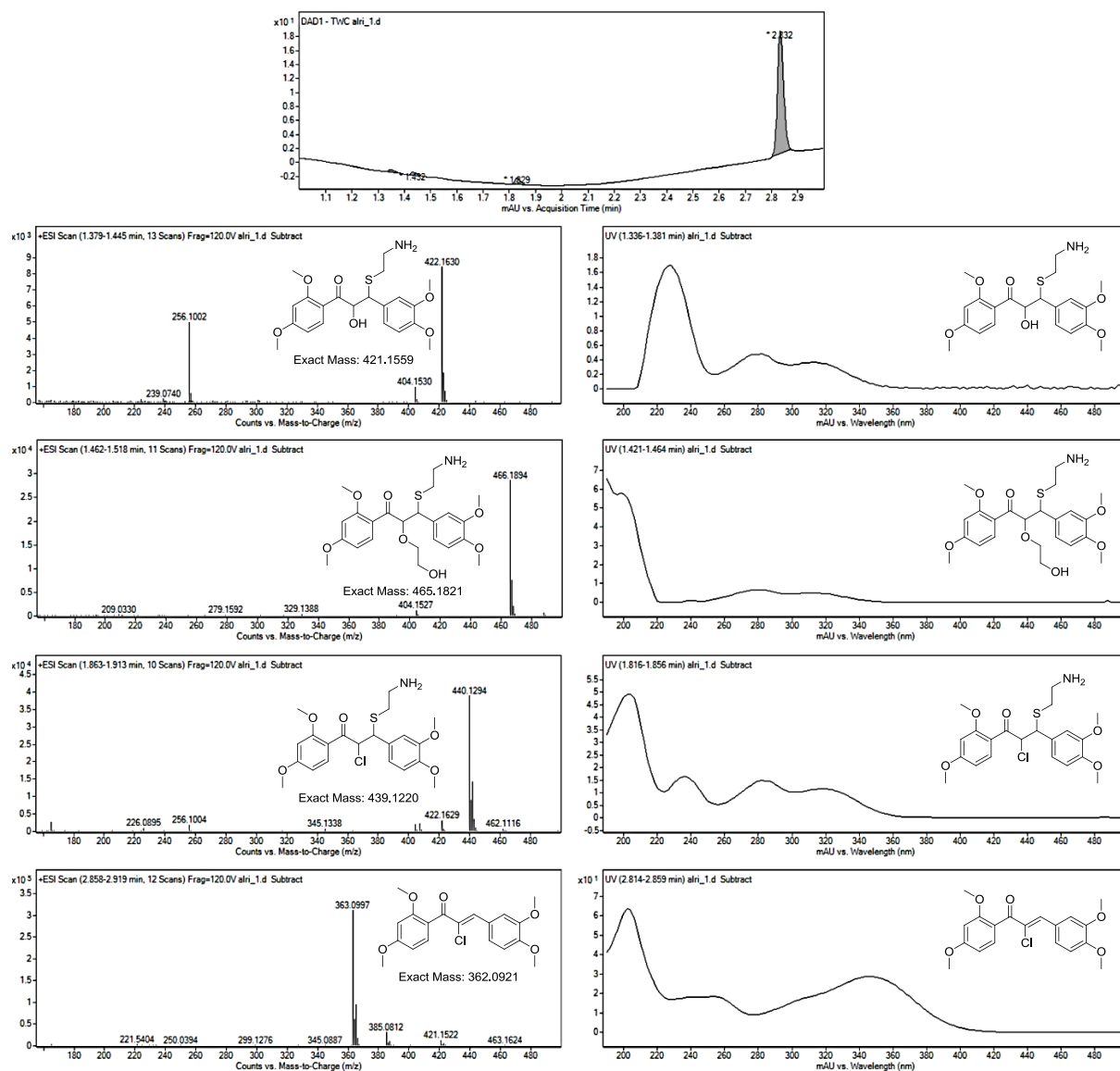
This measurement was done ~15 min after mixing the chalcone solution with the thiol solution. The measurement that was done directly after mixing showed the presence of the direct thiol adduct.



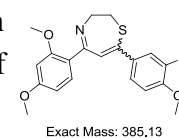
α -Br-TMC (73) with 60 fold 1,4-butanedithiol

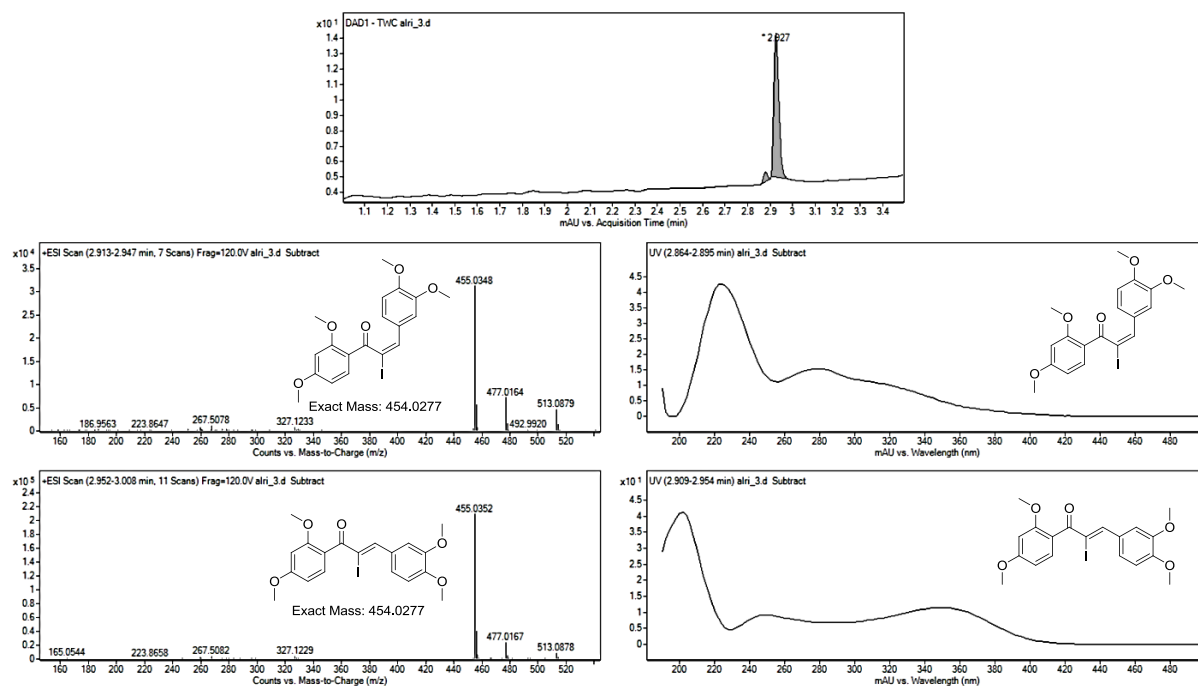
α -Br-TMC (73) with 60 fold 2-mercaptoethanol

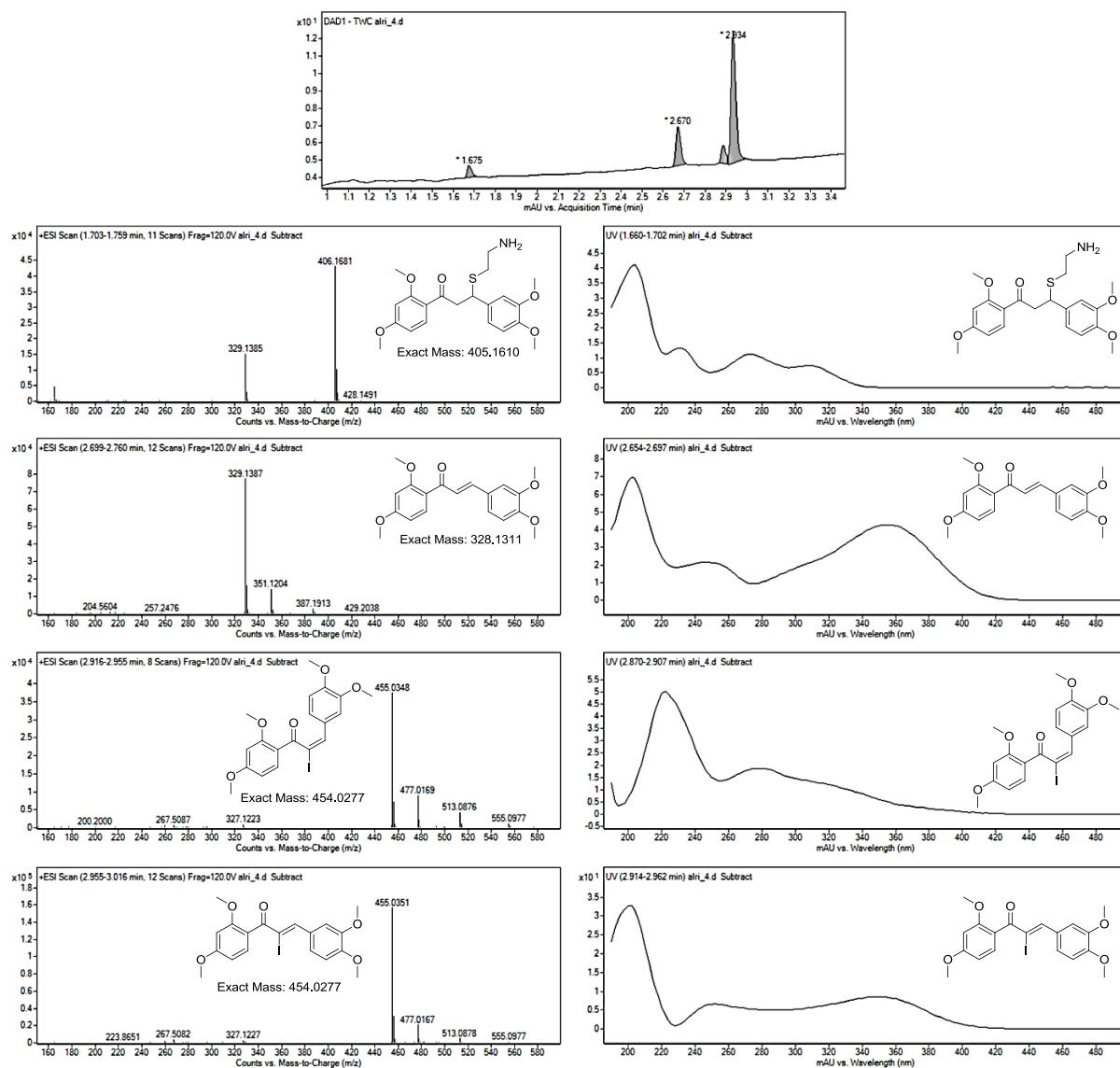
α -Br-TMC (73) with 60 fold glutathione

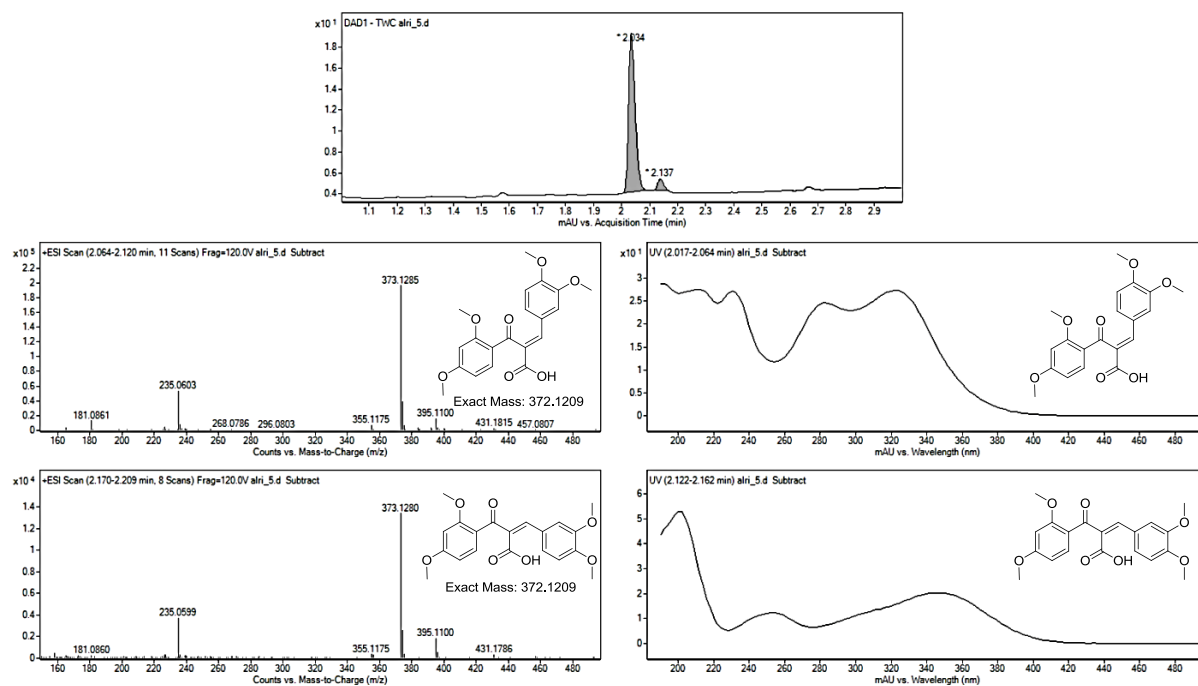
α -Cl-TMC (**74**) with 12 fold cysteamine

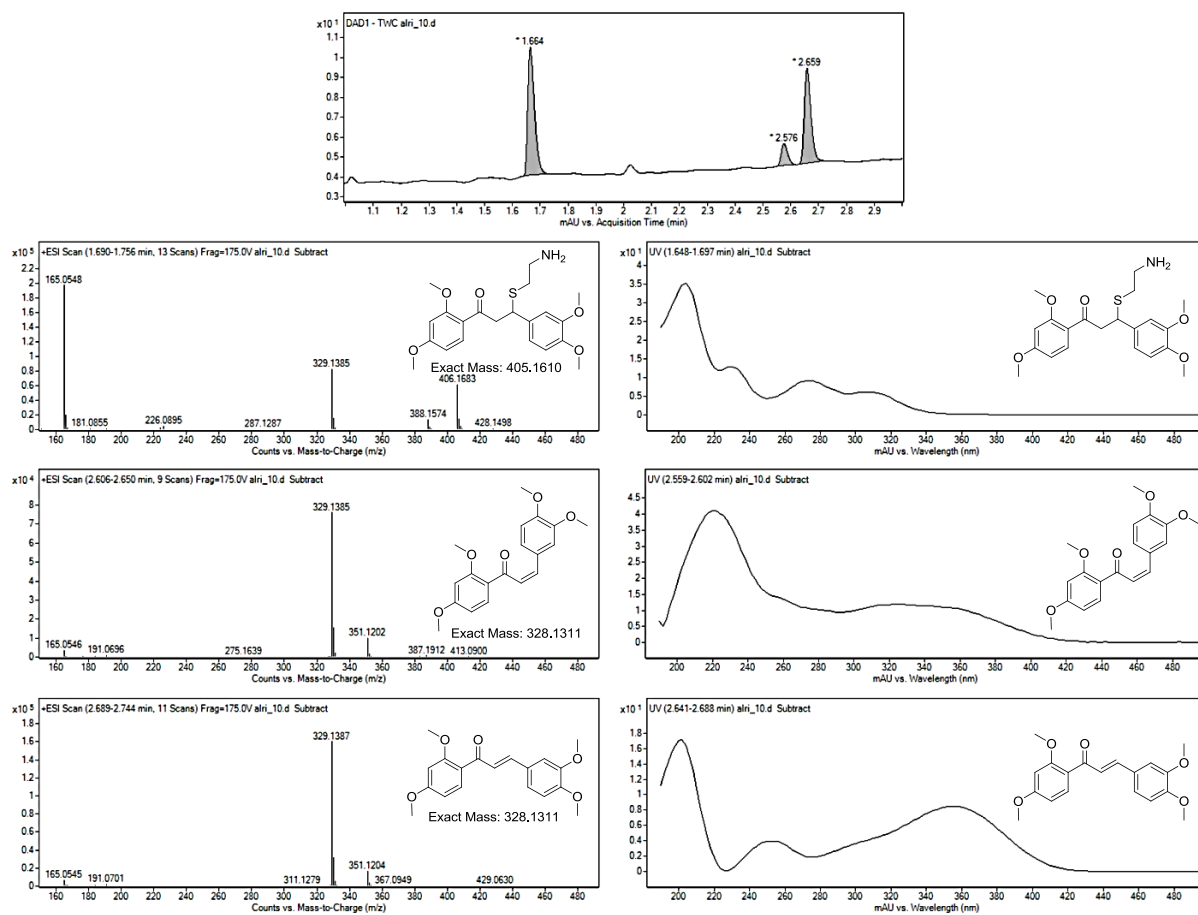
This measurement was done directly after mixing the chalcone solution with the thiol solution. When the measurement was repeated after about 24 h of incubation the major product was the thiazepine shown on the right (which was also detected in the case of α -Br-TMC (**73**) with cysteamine).

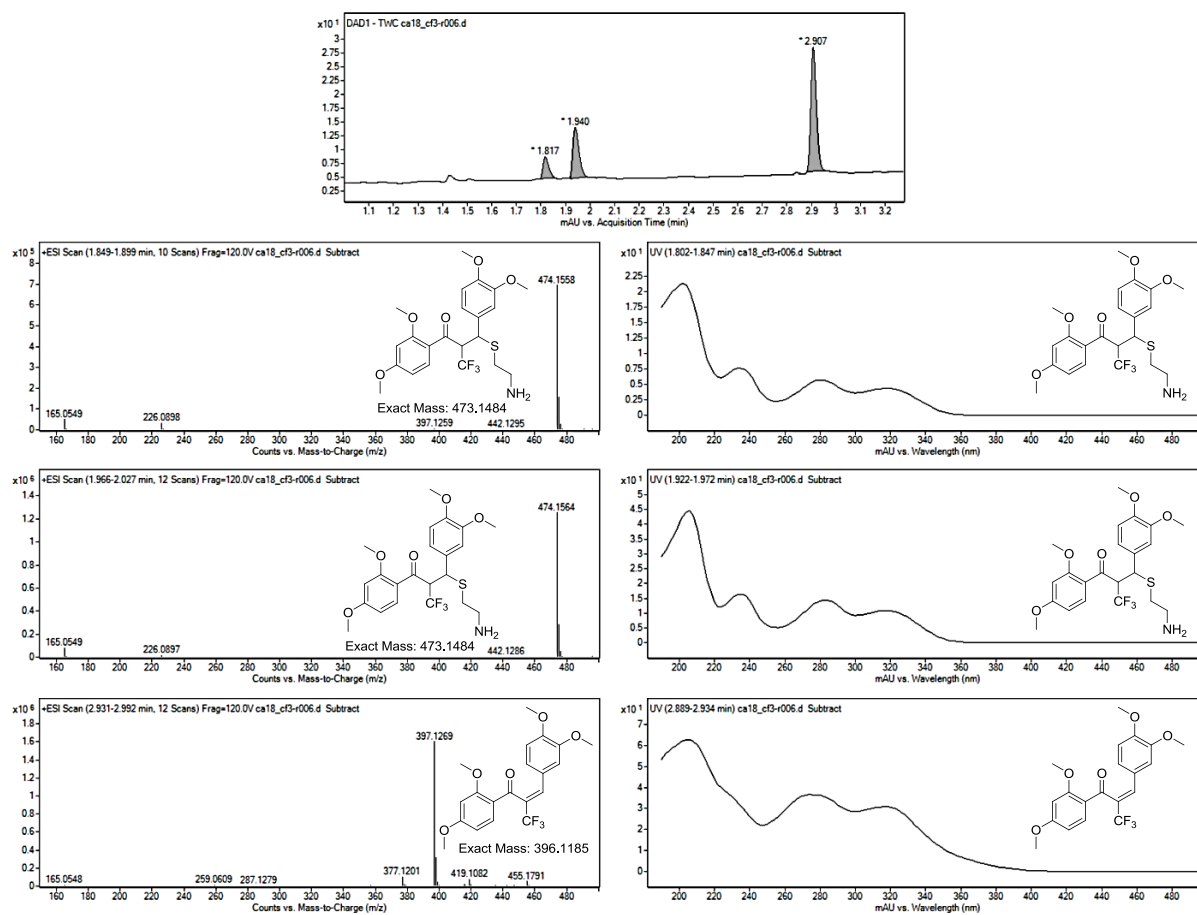


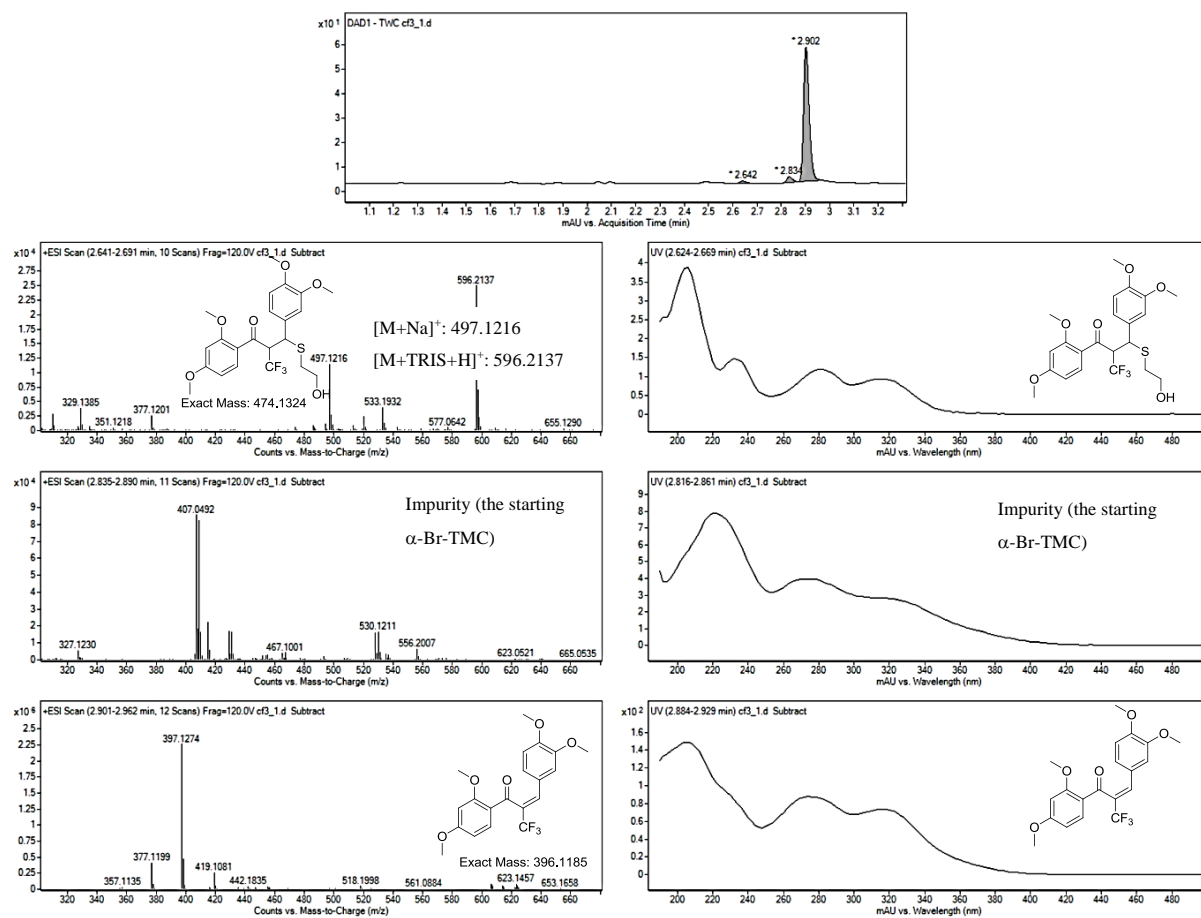
α -I-TMC (**75**) without thiol

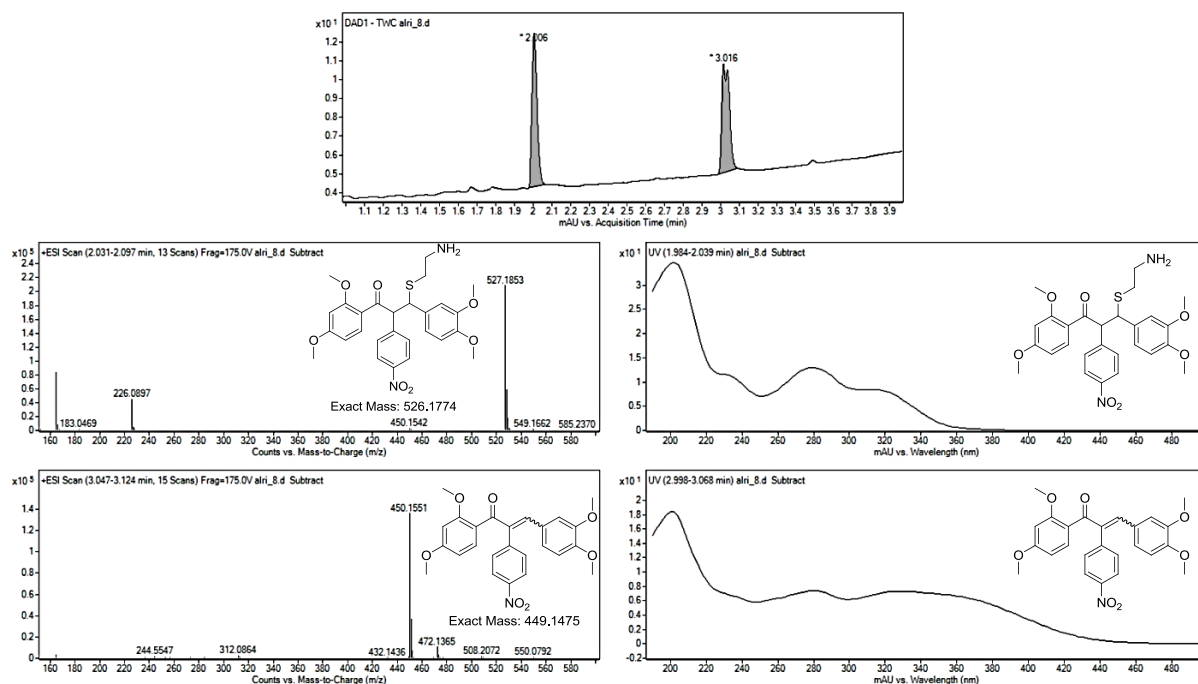
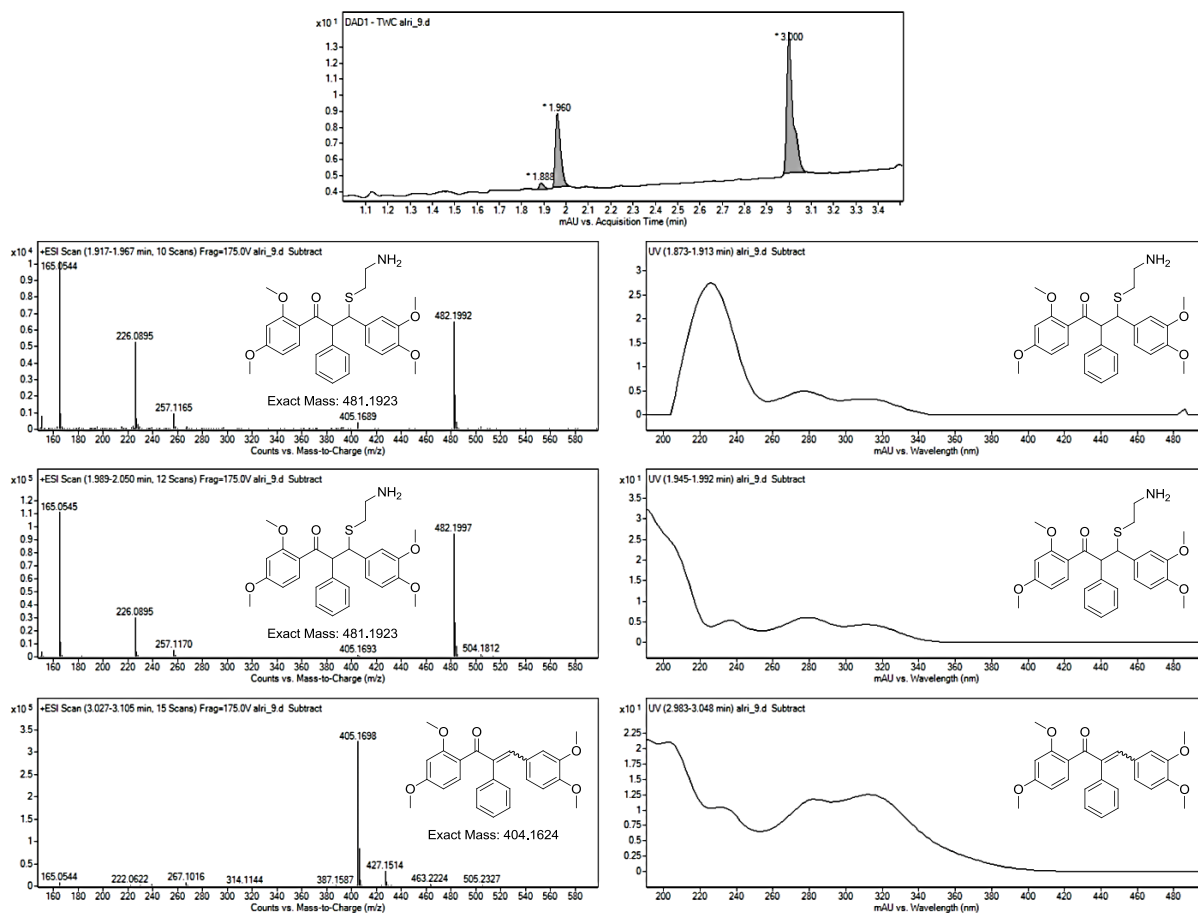
α -I-TMC (**75**) with 12 fold cysteamine

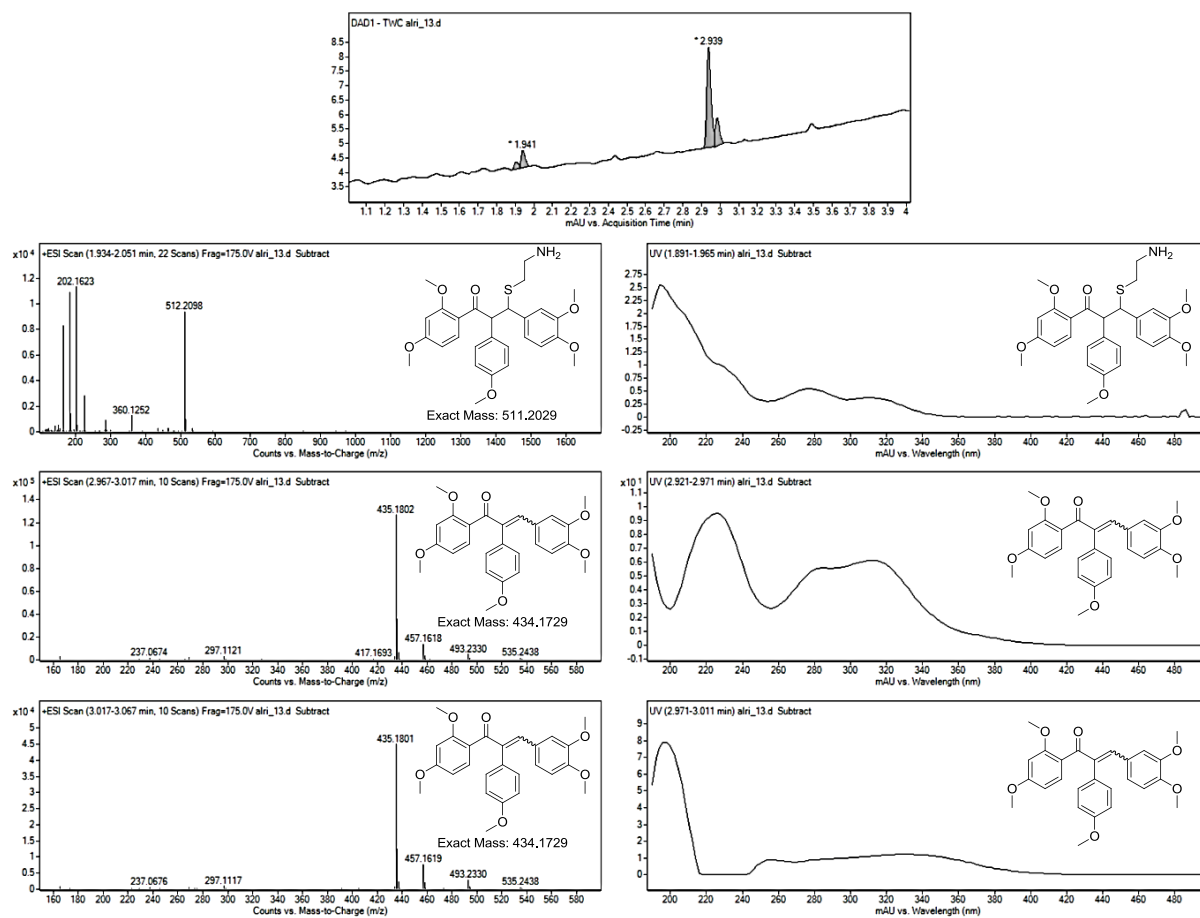
α -COOH-TMC (**76**) without thiol

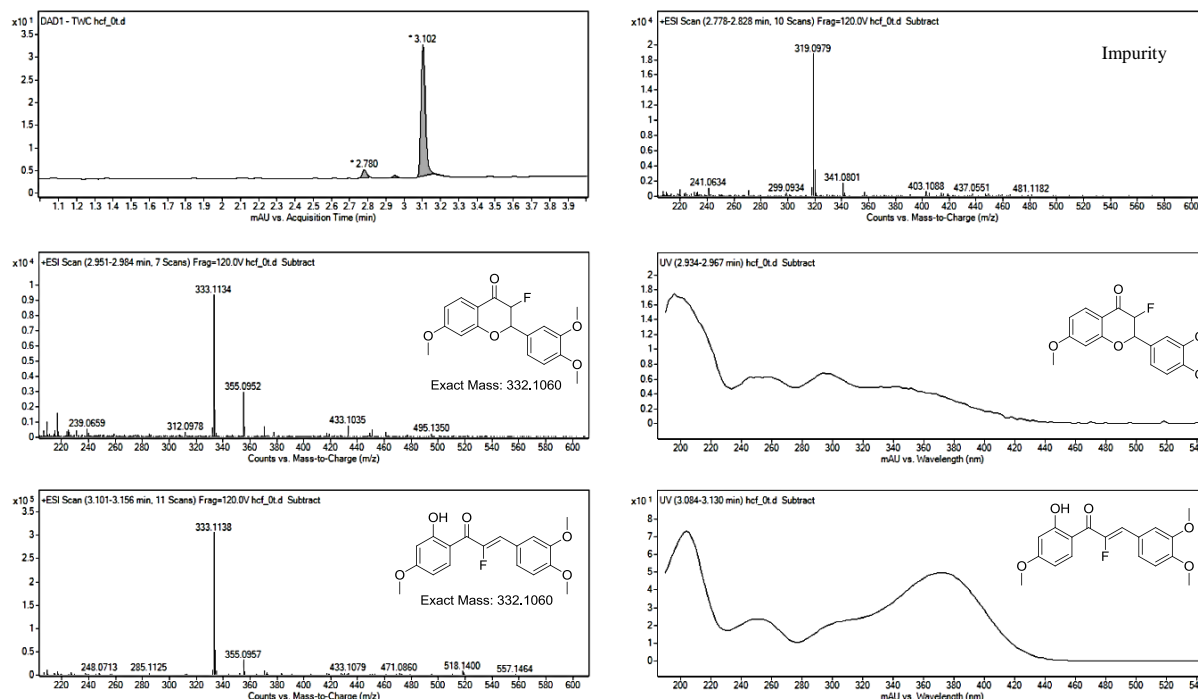
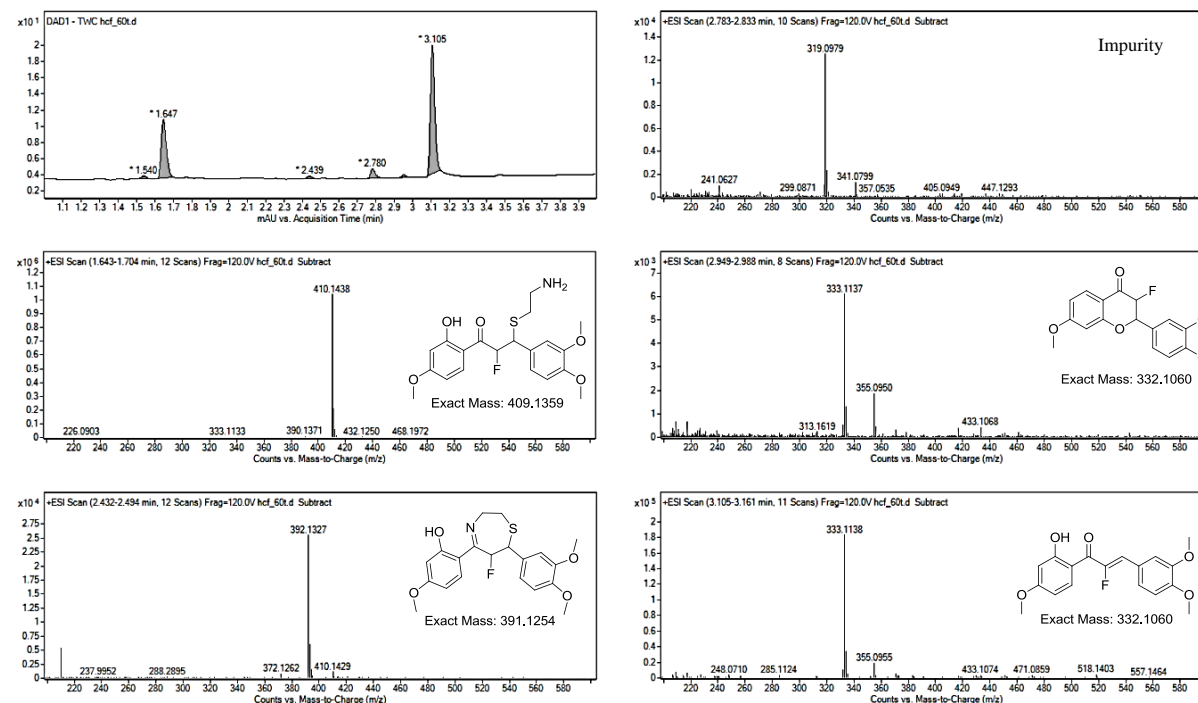
α -COOH-TMC (**76**) with 200 fold cysteamine

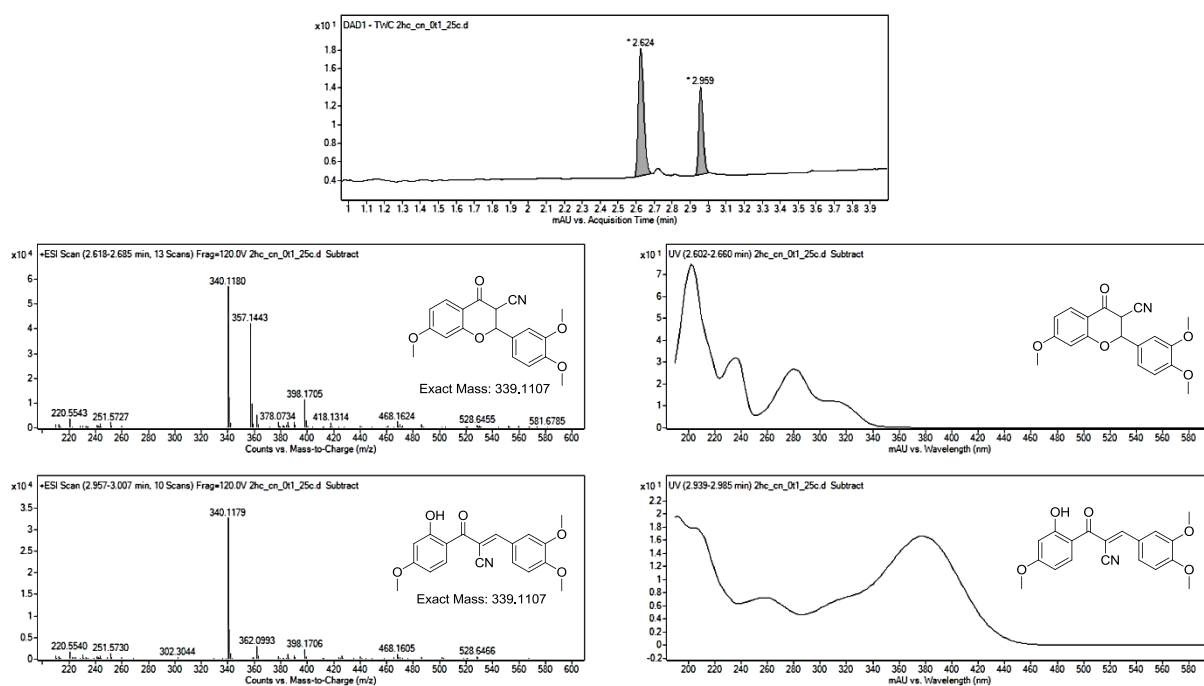
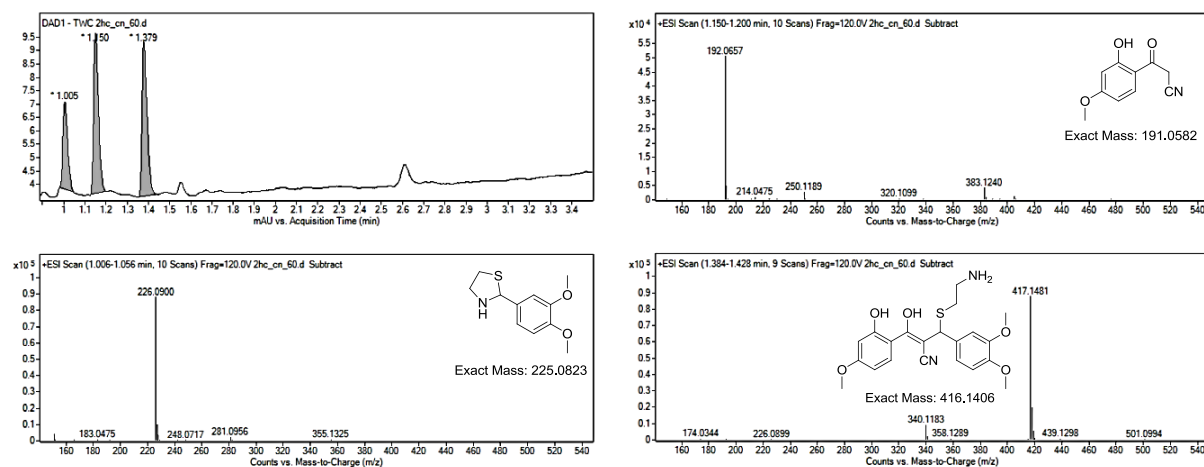
α -CF₃-TMC (**77**) with cysteamine

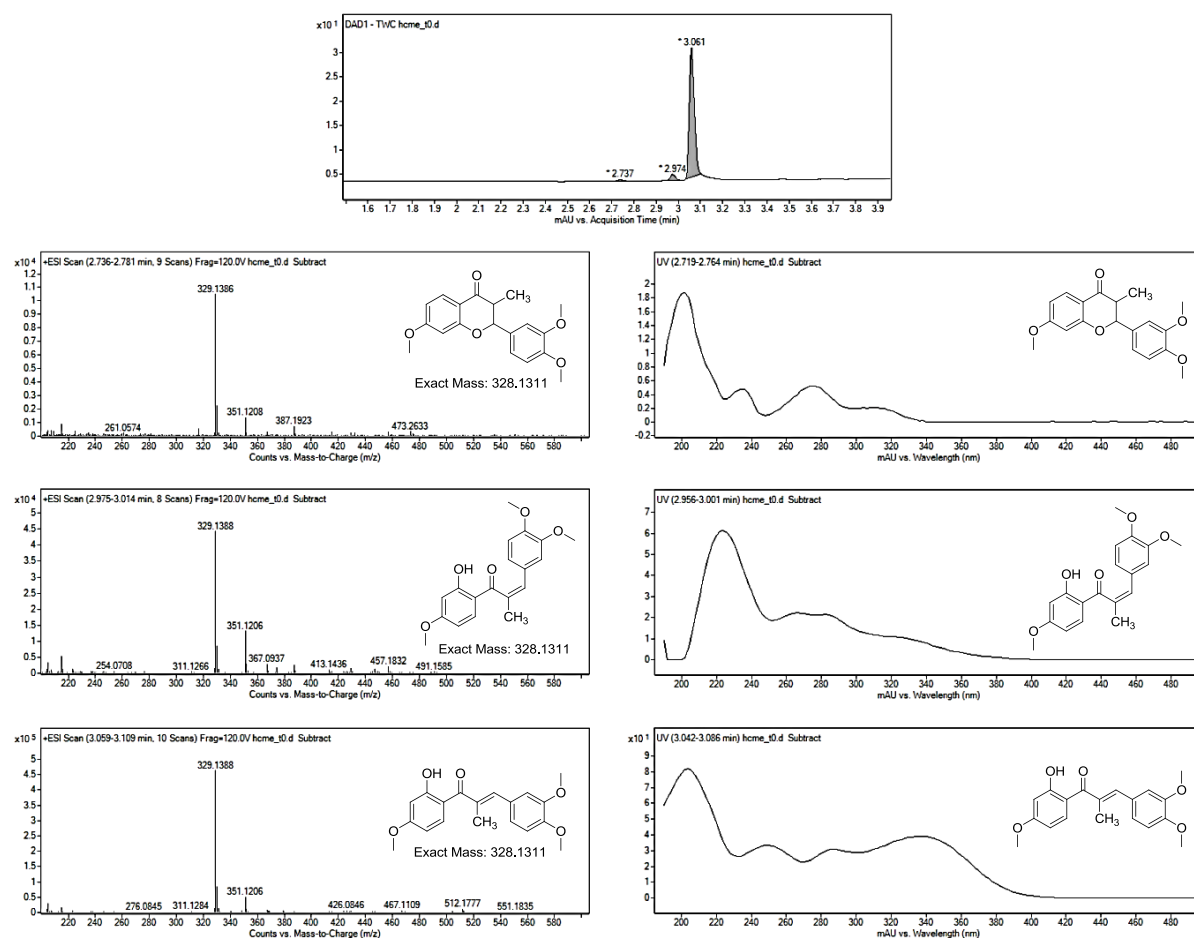
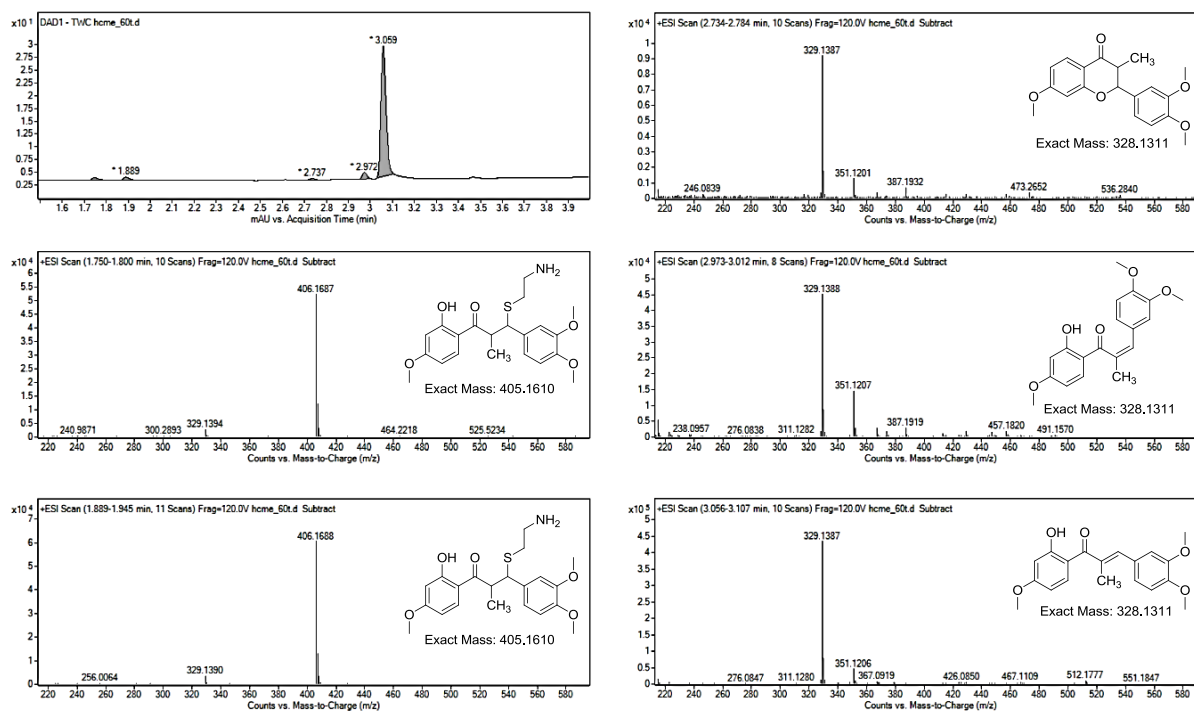
α -CF₃-TMC (77) with 2-mercaptoethanol

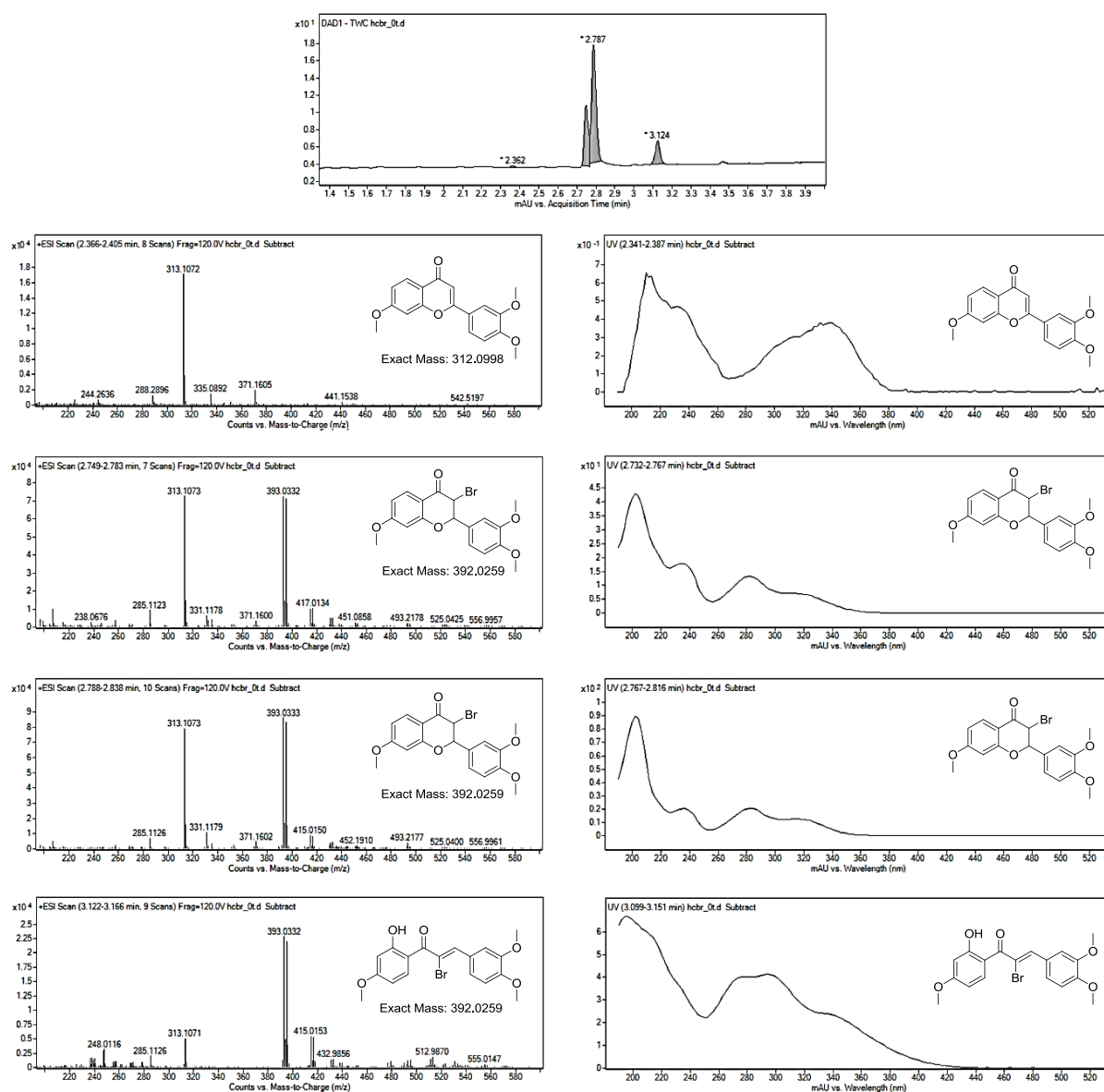
α -*p*-NO₂-C₆H₄-TMC (**78**) with 60 fold cysteamine α -Ph-TMC (**79**) with 200 fold cysteamine

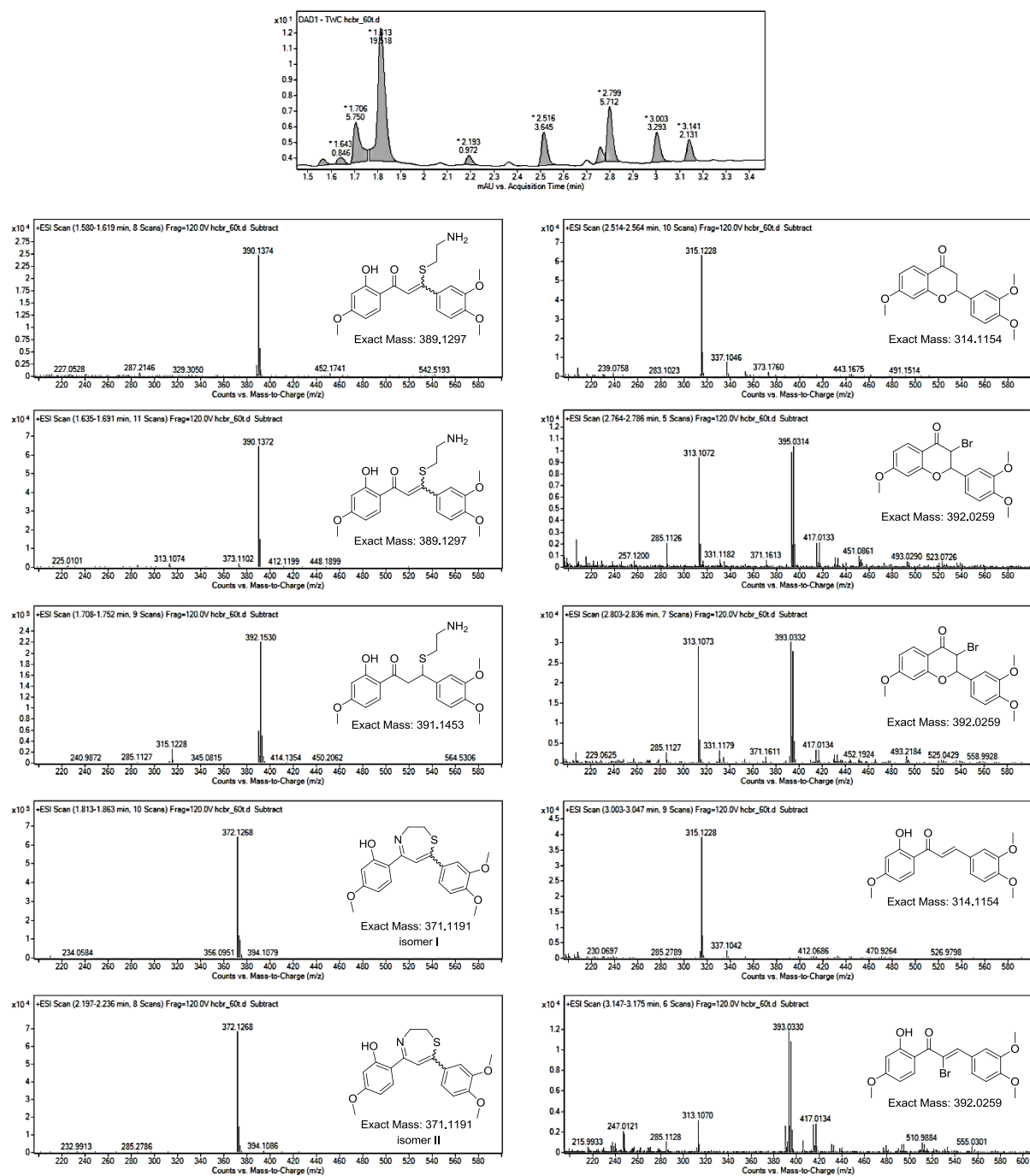
α -*p*-OMe-C₆H₄-TMC (**80**) with 100 fold cysteamine

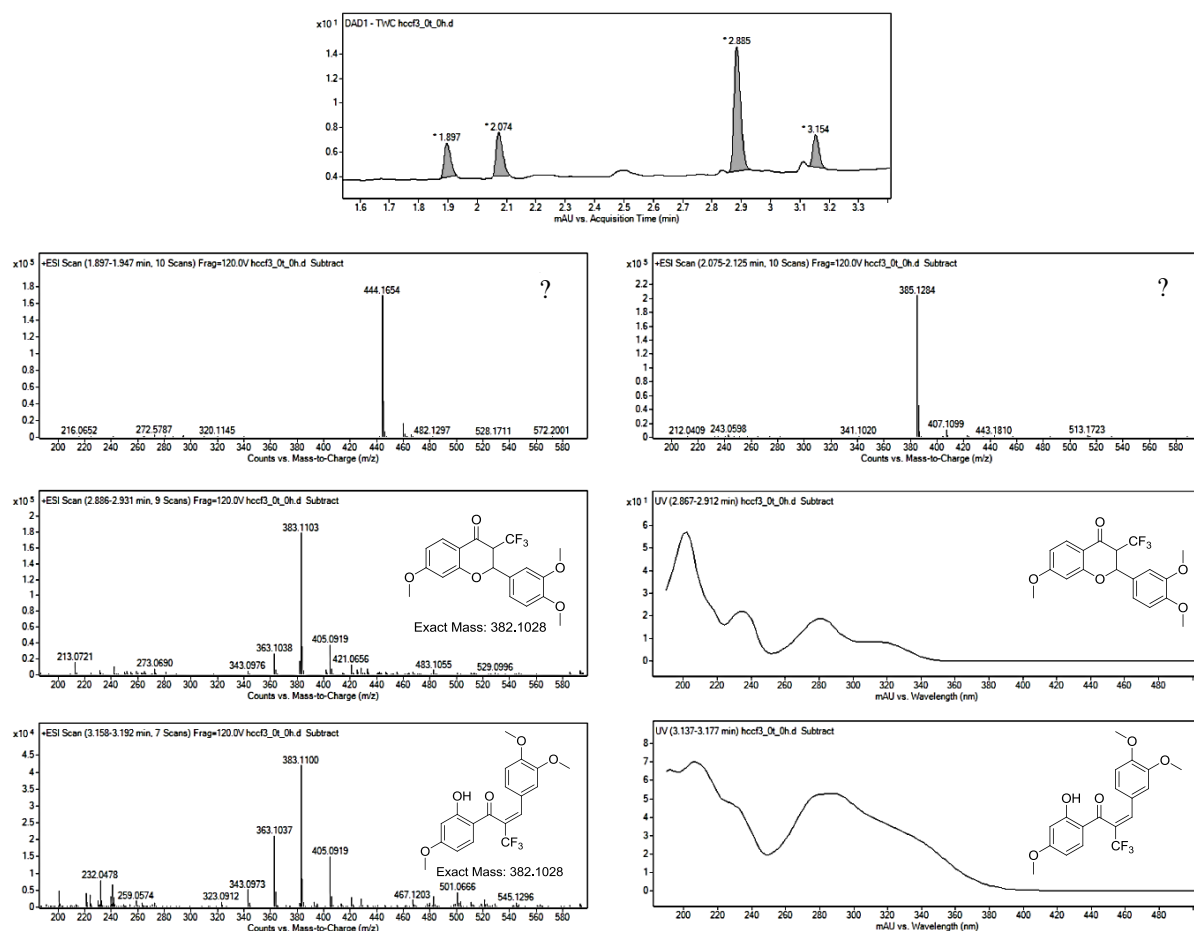
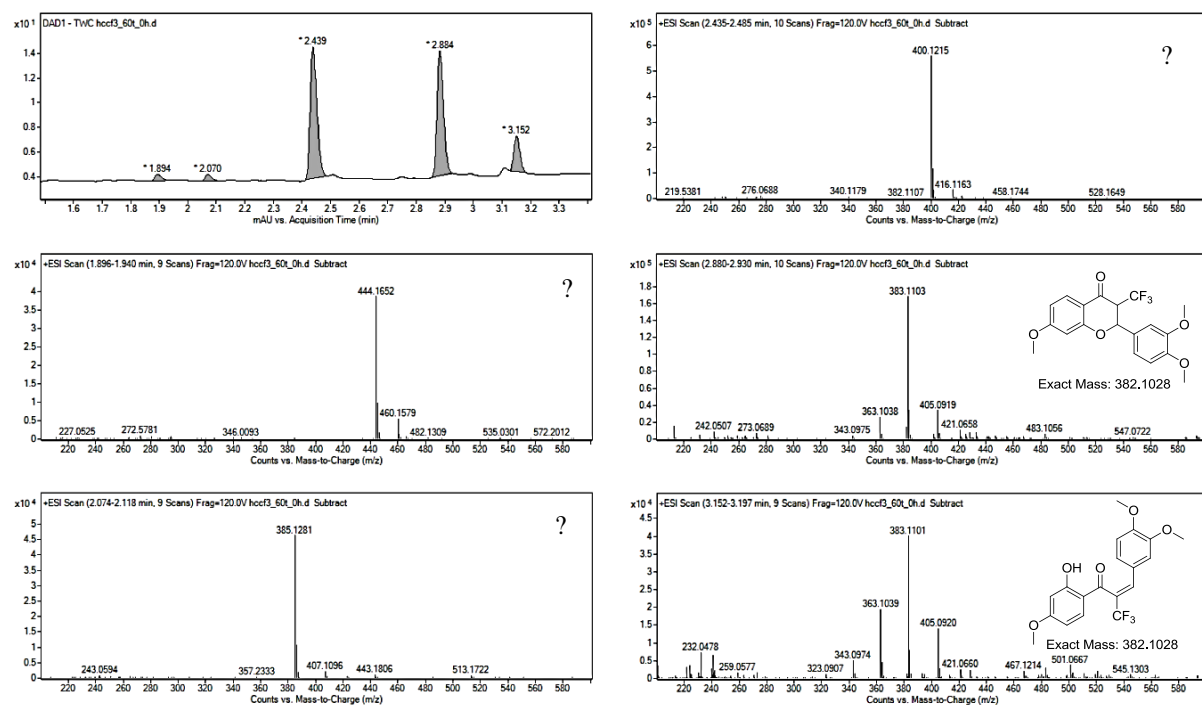
7.5.3. α -X-HCs in absence and presence of cysteamine α -F-HC (**94**) without thiol α -F-HC (**94**) with 60 fold cysteamine

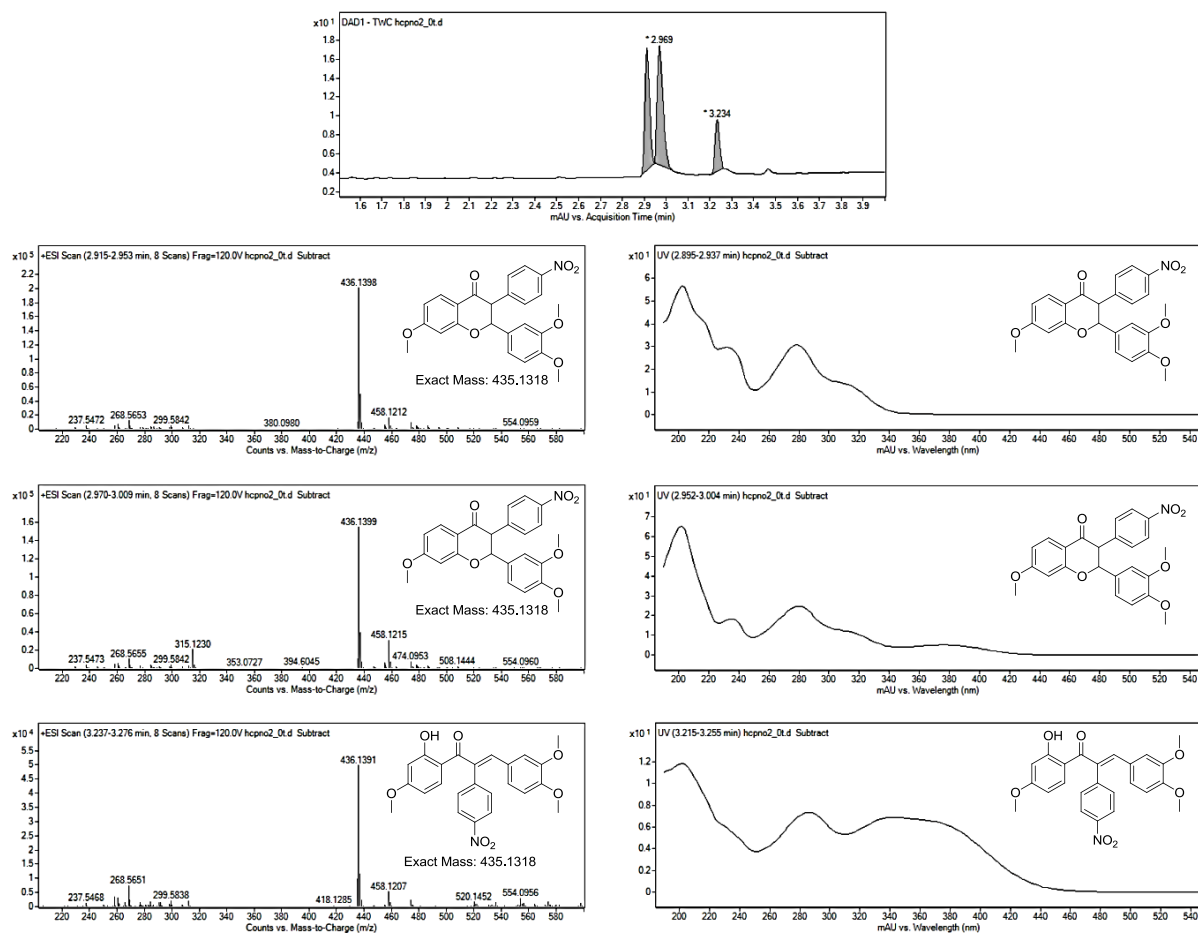
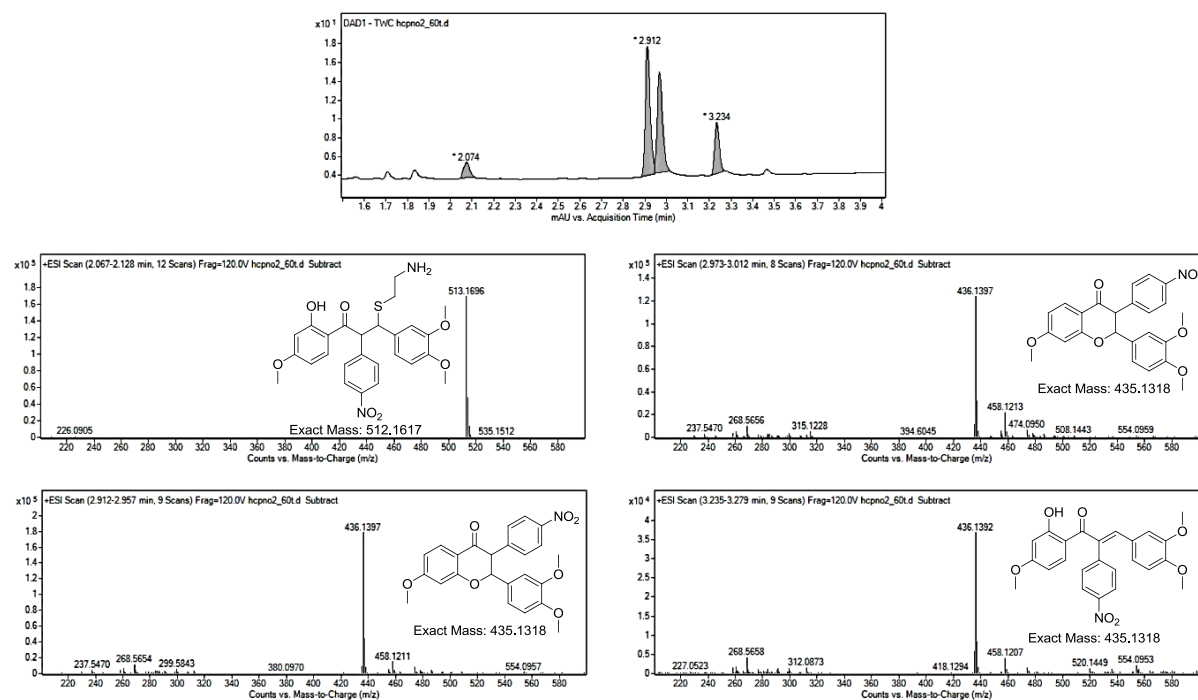
α -CN-HC (95) without thiol α -CN-HC (95) with 60 fold cysteamine

α -Me-HC (96) without thiol α -Me-HC (96) with 60 fold cysteamine

α -Br-HC (97) without thiol

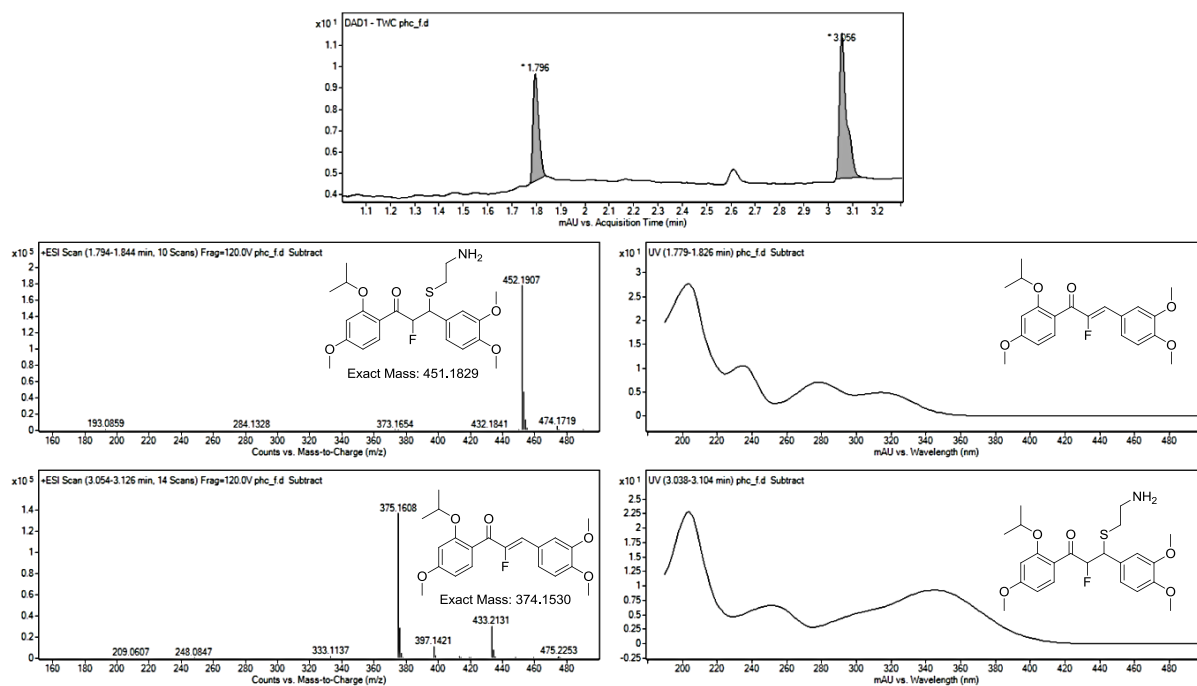
α -Br-HC (**97**) with 60 fold cysteamine

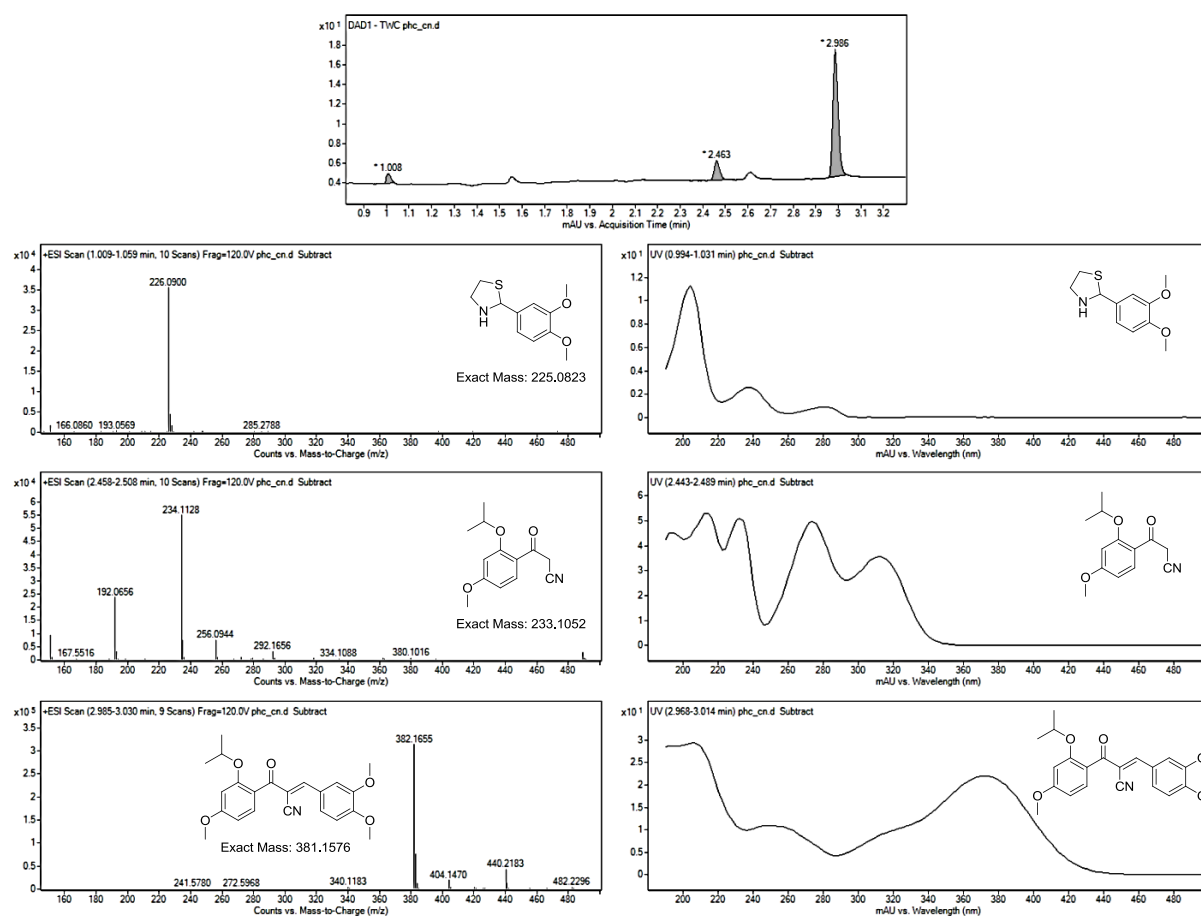
α -CF₃-HC (99) without thiol α -CF₃-HC (99) with 60 fold cysteamine

α -*p*-NO₂-C₆H₄-HC (**100**) without thiol α -*p*-NO₂-C₆H₄-HC (**100**) with 60 fold cysteamine

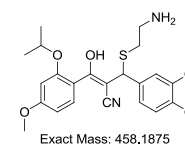
7.5.4. α -X-pHCs in presence of cysteamine

α -F-pHC (104) with cysteamine



α -CN-pHC (105) with cysteamine

This measurement was done like all the others with an HPLC run at 40 °C. When it was repeated at 25 °C the expected addition product shown on the right side was detected.



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CURRICULUM VITAE

Nafisah Al-Rifai

Born on 24.09.1982 in Anata-Jerusalem.

Professional Experience

Since Oct. 2010	A PhD student at Institut für Organische Chemie, Universität Regensburg (Regensburg, Germany). Supervisor: PD Dr. Sabine Amslinger. Thesis title: The α -Substitution of Chalcones as a Tool to Modulate the Reactivity and Biological Activity.
Apr. 2010 – Sep. 2010	Intensive German language course in InterDaF (Leipzig, Germany) - (DSH-2).
Feb. 2007 – Mar. 2010	Teaching and research assistant then lecturer at German-Jordanian University (Amman, Jordan).
Oct. 2004 – Feb. 2007	A Master student in Organic Chemistry, Chemistry Department at the University of Jordan (Amman, Jordan), excellent rating (3.94 out of 4). Supervisor: Prof. Musa Nazer (PhD from Harvard University). Thesis Title: Cyclization to Substituted-2 <i>H</i> -Indazoles: Study of the Reaction of 2-Nitro- <i>N</i> -substituted Benzylamines with Cyclic and Acyclic Amines. Involved in teaching Inorganic Chemistry, Organic Chemistry (I) and Organic Chemistry (II) laboratories at the department.
Oct. 2000 – Jun. 2004	A Bachelor student in Chemistry, Chemistry Department at the University of Jordan (Amman, Jordan), excellent rating (3.83 out of 4). The (1 st) out of (102) students in the Academic year 2003/2004. Involved in teaching Inorganic Chemistry and Organic Chemistry (II) laboratories, Chemistry Department at the University of Jordan during my B. Sc. studies (2003-2004).

Scholarship

Oct. 2010 – Mar. 2014	A PhD scholarship from DAAD (Deutscher Akademischer Austausch Dienst).
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Publications

1. Al-Gharabli, S.; Al-Rifai, N.; Saadeh, H. A.; Mosleh, I. M.; Mubarak, M. S., Solid phase synthesis and antiparasitic activity of a library of peptidyl metronidazoles. *Jordan Journal of Chemistry* **2010**, 5, 139-147.
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References

Dr. Sabine Amslinger
Universität Regensburg
Institut für Organische Chemie
Universitätsstraße 31
93053 Regensburg, Germany

Prof. Dr. Burkhard König
Universität Regensburg
Institut für Organische Chemie
Universitätsstraße 31
93053 Regensburg, Germany

Prof. Dr. Musa Nazer
University of Jordan
Faculty of Science, Chemistry Department
Amman, Jordan
E-mail: mzn06@yahoo.com

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